SOME PROPERTIES OF COMPRESSIONAL WAVES IN LENNARD-JONES AND DEVONSHIRE LIQUIDS

I. WEAK SOUND WAVES

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Summary

The present paper describes some calculations of the speed of sound u in a theoretical Lennard-Jones and Devonshire liquid. It is found that u decreases as the temperature is raised, but increases if the liquid is compressed. When quantum effects are considered it is found that u decreases as the value of de Boer's quantal parameter Λ^* is increased. All these effects have been observed in experiments on real liquids.

I. INTRODUCTION

The properties of compressional waves in a liquid are closely related to the forces acting between its molecules, and any theoretical treatment of such waves must start with a model for liquids which takes account of these forces. Kincaid and Eyring (1938) presented a treatment of this kind in which they assumed that the molecules are hard attracting spheres moving in a uniform potential field. The " smoothed " attractive forces between the molecules are represented by the uniform potential, and the repulsive forces are assumed to be zero except at the collision of molecules where they become infinite.

This is a crude model, but it was improved by Lennard-Jones and Devonshire (1937) who retained the simplification of spherical molecular symmetry but assumed that the molecules interact in pairs according to the more realistic function

$$\varepsilon=\varepsilon_0[r_0/r)^{12}-2(r_0/r)^6], \tag{1}$$

where ε is the interaction energy (relative to an energy zero at infinite separation) of two molecules whose centres are a distance r apart, and $-\varepsilon_0$ is the minimum value of ε which occurs at the separation $r=r_0$. The first term in this potential represents a repulsive force which predominates at small separations and the second represents an attraction which outweighs the repulsion at larger separations. Lennard-Jones and Devonshire further postulated that the molecules in a liquid spend most of their time near the sites of a close-packed cubic lattice. Each molecule is imprisoned in a cell bounded by its nearest neighbours but it can move classically within the cell subject to the forces between it and its neighbours. To simplify the mathematics, Lennard-Jones and Devonshire assumed that the potential energy of a molecule at a distance a from the centre of the cell can be taken as its average potential over the surface of the sphere of radius a, calculated on the supposition that the neighbouring molecules are

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fixed at their lattice sites. From these assumptions they derived a general equation of state (designated here, the " LJD " equation) which can be written in the reduced form

$$P^*=f(V^*,T^*), \tag{2}$$

where f is a complicated function and P^*, V^*, and T^* are dimensionless variables proportional to the pressure P, molar volume V, and temperature T, respectively. They are defined by

$$P^*=P/(2^{\frac{1}{2}}\varepsilon_0/r_0^3)=P/P_0, \tag{3}$$
$$V^*=V/(\mathbf{N}r_0^3/2^{\frac{1}{2}})=V/V_0, \tag{4}$$
$$T^*=T/(\varepsilon_0/\mathbf{k})=T/T_0, \tag{5}$$

where **N** is Avogadro's number and **k** is Boltzmann's constant (de Boer 1948). The molecular units P_0, V_0, T_0 have been listed for several liquids in an earlier paper (Hamann 1960).

It has been found that the LJD theory gives a fair qualitative description of the thermodynamics of simple fluids (see, for instance, de Boer and Lunbeck 1948 ; Wentorf *et al.* 1950 ; Rowlinson 1959) and we have considered it worthwhile to use it as a basis of some calculations of the behaviour of compressional waves in liquids.

The present paper will be concerned with the speed of weak (sound) waves and Part II of this series will consider the properties of strong (shock) waves.

II. Method of Calculation

(*a*) *General*

At low frequencies and low amplitudes the speed of sound u in a pure liquid is related to the thermodynamic properties of the substance by

$$u=V\left[\frac{T}{MC_V}\left(\frac{\partial P}{\partial T}\right)_V^2-\frac{1}{M}\left(\frac{\partial P}{\partial V}\right)_T\right]^{\frac{1}{2}}, \tag{6}$$

where M denotes the molar weight of the liquid and C_V is its molar heat capacity at constant volume. If the molecules interact according to the potential function (1), this relation can be written in the reduced form

$$u^*=V^*\left[\frac{T^*}{C_V^*}\left(\frac{\partial P^*}{\partial T^*}\right)_V^2-\left(\frac{\partial P^*}{\partial V^*}\right)_T\right]^{\frac{1}{2}}, \tag{7}$$

where P^*, V^*, and T^* are defined by (3), (4), and (5), and

$$C_V^*=C_V/\mathbf{Nk}, \tag{8}$$
$$u^*=u/(\mathbf{N}\varepsilon_0/M)^{\frac{1}{2}}=u/(\varepsilon_0/m)^{\frac{1}{2}} \tag{9}$$
$$=u/u_0,$$

m being the molecular mass. Some values of u_0 for several liquids have been listed previously (Hamann 1960).

For monatomic liquids or for simple diatomic liquids in which the molecular rotation is restricted, C_V^* can be calculated from the equation of state. It follows that u^* can also be derived from this equation.

(*b*) *Classical LJD Liquids*

In its original form the LJD theory assumed that the motion of each molecule within its cell obeys the laws of classical mechanics. This assumption is justifiable if the molecules are heavy (e.g. A, Kr, Xe, N_2) but it is invalid for light molecules (e.g. H_2, D_2, He^3, He^4, Ne). Here we shall consider the classical model first and then treat the quantal generalization as a correction to the classical theory.

Wentorf *et al.* (1950) have published extensive tables of the thermodynamic properties of classical LJD fluids. Amongst the properties they listed were the compressibility factor P^*V^*/T^* $(=PV/\mathbf{N}kT)$ and the heat capacity C_V^*, both as functions of V^* and T^*. Their data allow us to estimate the derivatives $(\partial P^*/\partial T^*)_V$ and $(\partial P^*/\partial V^*)_T$, and hence to derive u^* for a wide range of pressures, volumes, and temperatures.

In the liquid region of the LJD theory P^* changes quite slowly with T^* at constant volume, and $(\partial P^*/\partial T^*)_V$ can be found accurately by the method of differences. But at constant temperature P^* is a rapidly changing function of V^* and it is necessary to fit the tabulated values to an analytic expression in order to arrive at reliable values for $(\partial P^*/\partial V^*)_T$. By trial we have found that the polynomial

$$P^*=a+b/V^*+c/V^{*2}+d/V^{*3} \tag{10}$$

(at constant T^*) gives a good description of the P^*-V^* relation over the range of volumes V^* between 0·9899 and 1·5556, and at temperatures T^* between 0·7 and 1·0. We therefore fitted the data to this formula by least squares, using a standard programme for the SILLIAC computer, and then derived $(\partial P^*/\partial V^*)_T$ by straightforward differentiation.

(*c*) *Quantal LJD Liquids*

In liquids composed of light molecules it is not justifiable to assume that the molecules move classically within their cells: it is necessary to allow for the finite spacing of the energy levels. de Boer (1948) has shown in a general way that this correction makes the thermodynamic functions dependent on a quantal parameter Λ^* as well as on V^* and T^* (or P^* and T^*). The quantity Λ^* is defined by

$$\Lambda^*=2^{\frac{1}{2}}\mathbf{h}/r_0(m\varepsilon_0)^{\frac{1}{2}}, \tag{11}$$

where $\mathbf{h}$ is Planck's constant. It is the reduced de Broglie wavelength of relative motion of two molecules of mass m and relative kinetic energy ε_0, and it is a characteristic property of the molecules. The greater its value the more will the liquid deviate from classical behaviour. Values of Λ^* for some simple liquids have been listed in an earlier paper (Hamann 1960).

Several attempts have been made to generalize the LJD theory to allow for the influence of Λ^*. de Boer and Lunbeck (1948) worked out the quantum correction to P^* in the form of an infinite power series in Λ^{*2}, but unfortunately the series often fails to converge. Hamann (1952) proposed an alternative treatment which involved some physical and mathematical simplifications but had the advantage of giving the quantum correction in a simple closed form.

Recently Levelt and Hurst (1960) have presented an exact calculation based on numerical computations of the energy eigen values for molecular motion in the complex LJD field. But it is doubtful whether the work involved in these computations is justified by the crude nature of the original LJD model, and we have preferred here to use the approximate, but convenient, algebraic correction (Hamann 1952). In the reduced units, the correction to the pressure is (David and Hamann 1953).

$$\underset{\text{(quantal)}}{P^*} - \underset{\text{(classical)}}{P^*} = T^*\left(1+\frac{3}{2}\,\frac{V^*}{y^*}\,\frac{\mathrm{d}y^*}{\mathrm{d}V^*}\right)\Big/ V^*(x^*-1), \tag{12}$$

provided that $x^* \geqslant 1{\cdot}5$, where

$$x^* = 9{\cdot}071 y^{*\frac{1}{3}} T^{*\frac{1}{2}} V^{*\frac{1}{3}} \Lambda^{*-1}, \tag{13}$$

and y^* is related to V^* by

$$(1+12y^*+25{\cdot}2y^{*2}+12y^{*3}+y^{*4})/(1+y^*)(1-y^*)^6 - 2V^{*2} = 0. \tag{14}$$

The corresponding correction to the heat capacity is

$$\underset{\text{(quantal)}}{C_V^*} - \underset{\text{(classical)}}{C_V^*} = 3(x^*-2)/4(x^*-1)^2. \tag{15}$$

We have applied the correction (12) to the classical LJD pressures listed by Wentorf *et al.* (1950) and then calculated the derivatives $(\partial P^*/\partial T^*)_V$ and $(\partial P^*/\partial V^*)_T$ in the same way as before.

III. Results and Discussion

(*a*) *Liquids at Low Pressures*

If a liquid is at a temperature below its normal boiling point then its reduced vapour pressure P^* is very small and can be assumed to be zero. Under these conditions V^* and u^* depend only on T^* and Λ^* (Hamann 1960). We have used equation (10) to find the zero pressure values of V^* and applied equation (7) to calculate the corresponding values of the reduced speed of sound. The results are listed in Table 1.

It will be seen that u^* decreases with increasing temperature, in contrast to its behaviour in a perfect gas (Hamann 1960), and that at a particular temperature it decreases with an increase in the quantal parameter Λ^*. This last effect arises from the fact that the zero-point energy inflates the volume of the liquid and makes it much more compressible than a classical liquid.

The theoretical results are compared with experimental data in Figure 1. It is clear that the experiments show the predicted dependence of u^* upon T^* and Λ^* although the numerical agreement is not very good. The lack of agreement evidently arises from the faults of the " cell " model rather than from the mathematical approximations of the LJD theory. We find that Dahler and Hirschfelder's (1961) improved cell theory gives even worse agreement with experiment, the calculated values of u^* being about 20% higher than those for the LJD theory. Barker's (1961) new " tunnel " model gives good results for classical liquids but is not easily applied to quantal ones.

TABLE 1

SOME REDUCED PROPERTIES OF CLASSICAL AND "QUANTIZED" LJD LIQUIDS AT ZERO PRESSURE

LJD Liquids	Temperature T^*	Volume V^*	Coefficient of Thermal Expansion $\frac{1}{V^*}\left(\frac{\partial V^*}{\partial T^*}\right)_P$	Isothermal Compressibility $-\frac{1}{V^*}\left(\frac{\partial V^*}{\partial P^*}\right)_T$	Heat Capacity C_V^*	Speed of Sound u^*
	0	0·916	0	0·0133	0	8·30†
	0·70	1·037	0·244	0·0348	2·61	6·63
	0·75	1·050	0·261	0·0386	2·58	6·47
(*a*) Classical:	0·80	1·065	0·287	0·0433	2·55	6·34
$\Lambda^*=0$	0·85	1·081	0·316	0·0493	2·53	6·17
	0·90	1·099	0·352	0·0571	2·50	5·98
	0·95	1·120	0·405	0·0683	2·47	5·78
	1·00	1·145	0·491	0·0851	2·43	5·60
	0·70	1·090	0·319	0·0454	2·71	6·00
	0·75	1·109	0·358	0·0527	2·68	5·77
(*b*) Quantal:	0·80	1·130	0·408	0·0626	2·64	5·52
$\Lambda^*=0·5$	0·85	1·155	0·477	0·0771	2·59	5·24
	0·90	1·186	0·584	0·1004	2·54	4·92
	0·95	1·226	0·784	0·1458	2·47	4·53
	1·00	1·290	1·366	0·286	2·38	3·99
	0·70	1·213	0·474	0·0819	2·46	4·87
(*c*) Quantal:	0·75	1·245	0·586	0·1061	2·47	4·58
$\Lambda^*=1·0$	0·80	1·288	0·780	0·1516	2·45	4·22
	0·85	1·353	1·287	0·280	2·38	3·71

† This value was derived previously (Hamann 1960).

Our calculations on quantal liquids were limited to values of Λ^* less than 1·5 for two reasons:

(i) If Λ^* is much greater than one, the stable range of the liquid state is shifted to lower reduced temperatures than are covered by the tables of Wentorf *et al.* (1950).

(ii) Equations (12) and (15) were based on an Euler–Maclaurin expansion of the partition function (Hamann 1952) which is only valid when x^* is greater than 1·5. If Λ^* is large, x^* becomes less than this value. We have therefore not been able to apply the theory directly to H_2 and the helium isotopes, but the trend of the curves to $\Lambda^*=1$ is certainly sufficient to explain the behaviour of the lighter liquids.

(*b*) *Liquids at High Pressures*

The computation of u^* is easily extended to compressed liquids. Using the polynomial form (10) of the P^*-V^* relation, we can derive values of the derivative $(\partial P^*/\partial V^*)_T$ over a wide range of temperatures and densities. As before, the derivative $(\partial P^*/\partial T^*)_V$ and the corresponding values of P^* and C_V^* can be taken directly from the tables of Wentorf *et al.* (1950). The results are

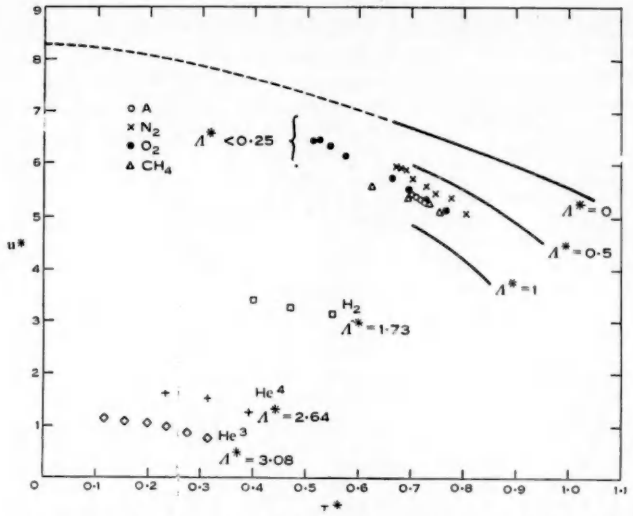

Fig. 1.—A comparison of the calculated and experimental speeds of sound in simple liquids. The sources of the experimental data for A, N_2, O_2, CH_4, H_2, and He^4 have been given in an earlier paper (Hamann 1960). The data for He^3 have been taken from a paper by Atkins and Flicker (1959).

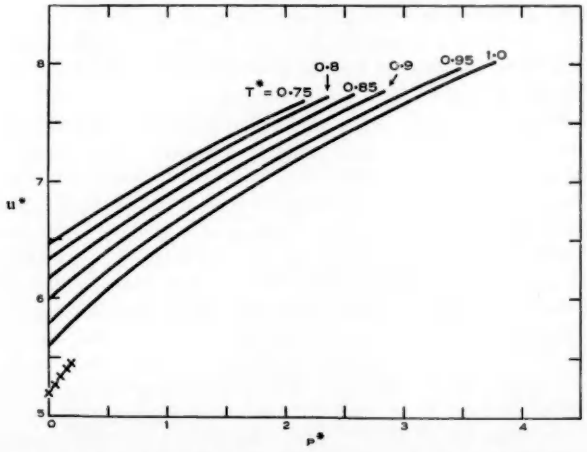

Fig. 2.—The effect of pressure on the speed of sound. The curves represent the theoretical Leonard-Jones–Devonshire relations and the crosses denote the experimental data for argon at $T^*=0\cdot75$.

plotted in Figure 2, which also shows some experimental data for liquid argon, obtained by van Itterbeek, van Dael, and Grevendonk (1959) in the pressure range 1 to 72 atm. Again the theory predicts the right kind of trend in u^* with increasing pressure. The agreement between theory and experiment would probably be better at higher pressures ($P^*>2$; that is, $P>1000$ atmospheres for argon), where the cell model becomes a more realistic one. There is a clear need for some experiments in this pressure range.

(c) *Rao's (1940) Relation*

Rao (1940) found empirically that the thermal coefficient of the speed of sound in many liquids is close to three times the thermal coefficient of the density. Expressed in a reduced form this relation becomes

$$\frac{1}{u^*}\left(\frac{\partial u^*}{\partial T^*}\right)_P = -A\frac{1}{V^*}\left(\frac{\partial V^*}{\partial T^*}\right)_P, \tag{16}$$

where $A\approx 3$. Later Carnevale and Litovitz (1955) observed that a parallel relation applies if the density is changed, not by temperature, but by pressure: that is

$$\frac{1}{u^*}\left(\frac{\partial u^*}{\partial P^*}\right)_T = -A'\ \frac{1}{V^*}\left(\frac{\partial V^*}{\partial P^*}\right)_T \tag{17}$$

where again $A'\approx 3$. These relations are very simple and we considered it worthwhile to see whether they have any basis in the LJD theory.

We find that for classical LJD liquids in the temperature range $T^*=0{\cdot}7$ to $1{\cdot}0$, the relation (16) fails rather badly. The factor A is only about $1{\cdot}7$ and it decreases with increasing temperature. On the other hand, the relation (17) is obeyed quite accurately. A' has the value $2{\cdot}7\pm 0{\cdot}1$ and is independent of both the temperature and the pressure, to at least $P^*=2{\cdot}5$ (equivalent to an absolute pressure of 1000 atm in liquid argon).

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THE ELIMINATION REACTION OF VICINAL DISULPHONYLOXY COMPOUNDS WITH SODIUM IODIDE

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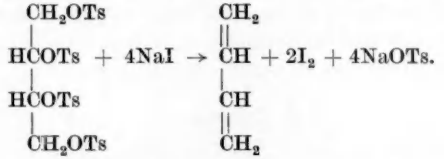
Summary

The elimination of two tosyloxy groups from vicinal ditosyloxy compounds by reaction with sodium iodide has been investigated. The reaction involves nucleophilic displacement of one tosyloxy group; this is followed by the simultaneous elimination of the second tosyloxy group and iodine, if the steric arrangement is favourable; if it is unfavourable, a second displacement by an iodide ion probably occurs to give substituents having a *trans*-arrangement suitable for elimination.

In one case, that of the ditosyl derivative of *trans*-cyclohexane-1,2-diol, the intermediate *cis*-2-iodocyclohexyl toluene-*p*-sulphonate was isolated.

I. INTRODUCTION

Sulphonyloxy groups, when attached to saturated carbon atoms, behave like halogen atoms toward nucleophilic reagents. Thus, a vicinal disulphonyloxy compound reacts with iodide ion by elimination of the sulphonyloxy groups with the establishment of an ethylenic linkage and the formation of molecular iodine. Tipson and Cretcher (1943) were the first to recognize this reaction clearly when they found that sodium iodide in acetone removed all four tosyloxy groups from 1,2,3,4-tetra-*O*-tosylerythritol, with the production of butadiene:



The reaction has since been used for the preparation of unsaturated sugar derivatives (for a review, see Tipson 1953) but in carbohydrate chemistry it was found applicable only to cases where one of the sulphonyloxy groups was primary. On the other hand, two secondary sulphonyloxy groups were eliminated from cyclitol derivatives by Angyal and Gilham (1958); the greater stability of cyclitols, compared to that of sugars, allowed here the use of more vigorous conditions.

Two applications of this reaction have been described, which possess stereochemical interest. Slates and Wendler (1955, 1956) applied the reaction to the 2,3-disulphonyloxy derivatives of an *allo*-steroid; they found that two of the diastereomers (the 2α,3β- and the 2α,3α-isomers) gave the Δ^2-olefin in good yield, whereas the other two (the 2β,3β and the 2β,3α) were recovered unchanged.

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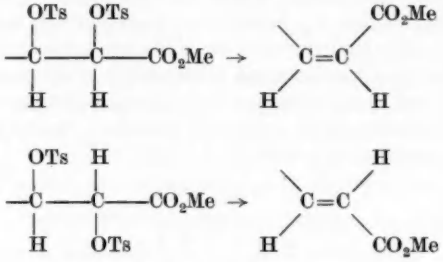
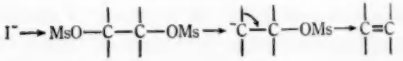
Previously Linstead, Owen, and Webb (1953) had shown that esters of *erythro*- and *threo*-αβ-dimesyloxy acids react with sodium iodide in acetone, with overall *cis*-elimination, to give the corresponding *cis*- and *trans*-unsaturated esters. This behaviour is opposite to that of the αβ-dibromo acids which undergo *trans*-elimination with sodium iodide:



In order to explain these cases of stereochemical specificity it seemed desirable to study the course of the reaction. Several suggestions have previously been advanced. Bladon and Owen (1950) proposed a course comprising independent displacement of each sulphonyloxy group by iodide ion to give a di-iodo compound which would be unstable and would decompose to iodine and an olefin. Foster and Overend (1951*b*) suggested an alternative mechanism in which the first step is the displacement (S_N2) by iodide ion of one sulphonyloxy group (a primary one, which is well known to undergo this reaction readily), followed by a concerted elimination (*E*2), of the remaining sulphonyloxy group and the iodine atom. In support of this suggestion these authors showed that 3,6-anhydro-1-deoxy-1-iodo-4,5-*O*-isopropylidene-2-*O*-tosyl-D-mannitol reacts more readily with sodium iodide in acetone than does the corresponding ditosyl compound. Newth (1956) produced evidence in support of this mechanism by showing that methyl 4,6-*O*-benzylidene-3-deoxy-3-iodo-2-*O*-tosyl-α-D-glucoside reacts quantitatively with sodium iodide in acetone in 10 min at 100 °C, whereas the corresponding ditosyl compound is unaffected by the same reagent during 24 hr at 100 °C. It is evident therefore that vicinal *trans*-iodosulphonyloxy compounds undergo elimination readily with sodium iodide; but it has not been proven that such a compound is an intermediate in the reaction of vicinal disulphonyloxy compounds with sodium iodide.

A completely different course for the reaction was proposed by James, Rees, and Shoppee (1955) to explain the results obtained by Slates and Wendler. They suggested that the reaction proceeds by the mechanism—designated as *ElcB* (Ingold 1953)—where the first step is the formation of a carbanion as a result of the removal of one sulphonyloxy group by combination with the iodide ion:



the anion then would lose a sulphonate ion to give the olefin.

II. The Reaction of Cyclic *cis*- and *trans*-Isomers

It is well known that the reaction of *trans*-1,2-dibromocyclohexanes with sodium iodide is much faster than that of the *cis*-isomers (Barton and Rosenfelder 1951; Alt and Barton 1954); this fact indicates the concerted nature of the former reaction. The ditosyloxycyclohexanes behave differently: preliminary experiments (Gilham 1956) have shown that the yield of sodium toluene-*p*-sulphonate is approximately the same, after a given time, from either the *cis*- or the *trans*-isomer. On repetition of these experiments it was observed, however, that the colour of iodine appeared much earlier in the reaction mixture containing the *cis*-isomer. It was then decided to determine the yield of sodium toluene-*p*-sulphonate (by filtration) and of iodine (by titration) formed from each isomer at intervals: the results are shown in Table 1.

Table 1

Reaction of 1,2-ditosyloxycyclohexanes with sodium iodide in acetone at 78 °C

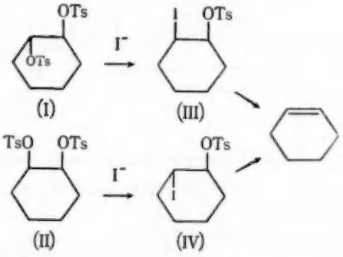
Time (hr)	*cis*-Isomer (II)		*trans*-Isomer (I)	
	NaOTs (moles)	Iodine (equiv.)	NaOTs (moles)	Iodine (equiv.)
4	0·20	0·19	0·14	0·06
8	0·31	0·27	0·26	0·16
16	0·61	0·50	0·58	0·34
32	1·02	0·77	1·04	0·71

The analysis for iodine is not accurate because iodine reacts slowly with acetone under the conditions of the experiment and is gradually removed thereby. The results in Table 1 nevertheless show that iodine is formed initially at the same rate as sodium toluenesulphonate from the *cis*-isomer (II), but at a much lower rate from the *trans*-compound (I). The reaction in the latter case therefore proceeds by a first step—in which iodine is *not* produced—giving an intermediate which releases iodine by a subsequent reaction. Formation of an intermediate in the reaction of the *trans*-compound is also shown by the fact that the starting material recovered (by dilution with water), after the reaction was allowed to proceed for some time, had a low melting point and was, therefore, contaminated. On the other hand pure starting material was recovered by the same procedure from the reaction of the *cis*-isomer indicating the absence of an intermediate in substantial amounts.

Subsequently, the intermediate was isolated in 10% yield from a 28-hr run of the *trans*-derivative (I), and gave correct analyses for an iodotosyloxycyclohexane. It was not identical with the known (Winstein, Grunwald, and Ingraham 1948) *trans*-2-iodocyclohexyl toluene-*p*-sulphonate (IV) and is therefore assigned the corresponding *cis*-structure (III). It reacts with sodium iodide in acetone, with the formation of equimolecular amounts of iodine and sodium toluenesulphonate, approximately five times faster than the ditosyl compounds (I) and (II). The *trans*-iodo compound (IV), on the other hand, reacts very rapidly

with sodium iodide even at room temperature (Winstein, Grunwald, and Ingraham 1948), obviously by a concerted mechanism.

Either of the two tosyloxy groups of the *trans*-isomer therefore react with iodide ion independently from each other by inversion to give the *cis*-iodo-intermediate. The mechanism by which the *cis*-iodotosyloxy compound (III) reacts further with iodide ion is not known : probably either the tosyloxy group or the iodine atom is displaced by inversion, giving a *trans*-compound which can undergo *E*2 elimination with ease. It can be assumed that the *cis*-ditosyloxy compound behaves in the same way (the initial rates being similar) but here the intermediate *trans*-iodo derivative reacts so rapidly with iodide ion that it cannot be isolated ; appearance of iodine is therefore at the same rate as that of sodium toluenesulphonate. These results vindicate the mechanism suggested by Foster and Overend (1951*b*) but are incompatible with that proposed by James, Rees, and Shoppee (1955).



This stepwise course of the reaction clearly explains its stereochemical outcome in the aliphatic series, as exemplified by the dimesyloxy compounds of Linstead, Owen, and Webb (1953). After the displacement of one group by inversion, elimination will occur in that conformation in which the C—O and the C—I bonds are antiparallel ; the overall result is *cis*-elimination. It is to be expected that this stereochemical outcome of the reaction will be general in all compounds where rotation is not constricted. However, Slates and Wendler's (1956) experiments do not appear to be explained by this mechanism.

The reaction of the ditosyloxycyclopentanes with iodide ion was investigated in a similar manner ; the results are shown in Table 2. These compounds react more rapidly than the cyclohexane derivatives, a general phenomenon for S_N2 reactions in these cyclic systems (Brown, Fletcher, and Johannsen 1951). The reaction of the *trans*-isomer is distinctly slower, probably because backside attack of the iodide ion is hindered by the adjacent tosyloxy group. There is no lag in the appearance of iodine from the *trans*-ditosyloxy compound ; nevertheless it is assumed that *cis*-iodocyclopentyl toluene-*p*-sulphonate is an intermediate and that it reacts much faster with iodide ion (being a *cis*-derivative of cyclopentane) ; therefore it does not appear in substantial concentrations.

The ditosyl esters of the four camphane-2,3-diols (Angyal and Young 1959) were also investigated, because the ring system in these compounds is rigid and nucleophilic attack on the *endo*-substituents is strongly hindered. It was indeed

found that 2-*endo*,3-*endo*-ditosyloxycamphane was very resistant to attack by iodide ion, being recovered unchanged after 94 hr at 100 °C. Each of the other three isomers reacted with iodide ion with the formation of sodium toluene-*p*-sulphonate and an amount of iodine much less than theoretical. It appears that

TABLE 2

REACTION OF 1,2-DITOSYLOXYCYCLOPENTANES WITH SODIUM IODIDE IN ACETONE AT 78 °C

Time (hr)	*cis*-Isomer		*trans*-Isomer	
	NaOTs (moles)	Iodine (equiv.)	NaOTs (moles)	Iodine (equiv.)
0·5	0·34	0·31		
1	0·47	0·44		
2	0·76	0·74		
4	1·25	1·23	0·41	0·39
8	1·90	1·67	0·71	0·70
16	1·98	1·90	1·40	1·01

these reactions are not straightforward eliminations but may be accompanied by skeletal rearrangements. Attempts to isolate a camphane derivative from the reactions were unsuccessful.

The reaction of tosyloxy groups with iodide ion is often inconveniently slow. Following a suggestion by Tipson (1953), Angyal and Gilham (1958) have shown

TABLE 3

REACTION OF DISULPHONYL COMPOUNDS WITH SODIUM IODIDE IN ACETONE

Compound	Time (hr)	Temp. (°C)	Yield of Sodium Salt (%)
trans-Cyclohexane-1,2-diol di-*p*-nitrobenzenesulphonate	1·25	78	66·5
ditoluene-*p*-sulphonate	32	78	51·5
ditoluene-*p*-sulphonate	8	78	13
dimethanesulphonate	8	78	25
Camphane-2*endo*,3-*exo*-diol di-*p*-nitrobenzenesulphonate ..	1	78	79
ditoluene-*p*-sulphonate	2	100	70

that vicinal *p*-nitrobenzenesulphonyl groups are eliminated more rapidly by iodide ion than are tosyl groups. It was found that the di-*p*-nitrobenzenesulphonyl esters of *trans*-cyclohexane-1,2-diol and of camphane-2-*endo*,3-*exo*-diol also react much faster than the corresponding tosyl compounds (see Table 3). The dimesyl ester of *trans*-cyclohexane-1,2-diol was also found to react somewhat

faster than the corresponding ditosyl compounds; in other cases, however, the opposite had been reported (Foster *et al.* 1949; Foster and Overend 1951*a*; Angyal and Gilham 1958).

III. Experimental

All melting points are corrected.

(*a*) *Preparation of the Disulphonyl Compounds.*—(i) *trans*-1,2-Ditosyloxycyclohexane, m.p. 110·5–111·5 °C, and its *cis*-isomer, m.p. 129 °C, were prepared according to Criegee and Stanger (1936); the *cis*- and *trans*-1,2-ditosyloxycyclopentanes, m.p. 89–90 °C and 109·5 °C, respectively, according to Owen and Smith (1952); *trans*-1,2-dimethanesulphonyloxycyclohexane, m.p. 132·5–133·5 °C, according to Clarke and Owen (1949).

(ii) trans-1,2-*Di*-p-*nitrobenzenesulphonyloxycyclohexane.* *trans*-Cyclohexane-1,2-diol (1 g) and *p*-nitrobenzenesulphonyl chloride (5·8 g), dissolved in anhydrous pyridine (10 ml), were allowed to stand at room temperature for 20 hr. Addition of ice precipitated a solid which was crystallized from ethanol to give needles of the *diester* (2·16 g, 51%), m.p. 147·5 °C (Found: C, 44·5; H, 3·5%. Calc. for $C_{18}H_{18}O_{16}N_2S_2$: C, 44·5: H, 3·7%).

(iii) *The 2,3-Ditosyloxycamphanes.* Each of the four camphane-2,3-diols (Angyal and Young 1959) (0·3 g) was allowed to stand for 2 weeks with toluene-*p*-sulphonyl chloride (1·1 g) in anhydrous pyridine (3 ml). Crushed ice was added and the precipitated solid was crystallized from methanol. The 2-*endo*,3-*exo*-diol gave an oily product which was extracted by chloroform; evaporation of the solvent left an oil which crystallized from light petroleum (b.p. 40–60 °C). Thus were obtained: (−)-2-exo,3-exo-*ditosyloxycamphane*, m.p. 139·5–140·5 °C, $[\alpha]_D^{20}$ −21° (*c*, 1·1 in chloroform) (Found: C, 60·4; H, 6·1%. Calc. for $C_{24}H_{30}O_6S_2$: C, 60·2; H, 6·3%); the (+)-2-endo,3-endo-*isomer*, m.p. 166·5–167·5 °C, $[\alpha]_D^{20}$ +42·6° (*c*, 1·1 in chloroform) (Found: C, 60·5; H, 6·2%); the (+)-2-exo,3-endo-*isomer*, m.p. 99·5–100·5 °C (decomp.), $[\alpha]_D^{20}$ +15° (*c*, 1·2 in chloroform) (Found: C, 60·5; H, 6·2%); and the (+)-2-endo,3-exo-*isomer*, m.p. 92–93 °C (decomp.), $[\alpha]_D^{20}$ +15·3° (*c*, 1·5 in chloroform) (Found: C, 60·6; H, 6·4%). The yield was about 90% in each case.

(iv) (+)-2-endo,3-exo-*Di*-p-*nitrobenzenesulphonyloxycamphane.* (+)-Camphane-2-*endo*,3-*exo*-diol (1 g) and *p*-nitrobenzenesulphonyl chloride (3·9 g), dissolved in anhydrous pyridine (10 ml), were allowed to stand at room temperature for 8 days. Crushed ice was added and the precipitated solid material was crystallized from acetone–methanol to give needles of the *camphane diester* (1·87 g, 59%), m.p. 121 °C (decomp.), $[\alpha]_D^{20}$ +25·3° (*c*, 0·6 in chloroform) (Found: C, 48·5; H, 4·75%. Calc. for $C_{22}H_{24}O_{16}N_2S_2$: C, 48·9; H, 4·5%).

(*b*) *Reactions with Sodium Iodide.*—(i) *Cyclohexane and Cyclopentane Derivatives.* Sulphonyl compound (0·212 g of the ditosyloxycyclohexanes, 0·103 g of the ditosyloxycyclopentanes, 0·136 g of the dimethanesulphonate, and 0·243 g of the di-*p*-nitrobenzenesulphonate of *trans*-cyclohexane-1,2-diol), sodium iodide (0·75 g), and anhydrous acetone (5 ml) were heated (in a sealed tube) immersed in the vapours of boiling ethanol. After intervals, the tubes were cooled in a dry ice-ethanol mixture and opened. After warming to room temperature to redissolve the precipitated iodine, the sodium salt was collected by filtration, washed with acetone (5 ml), dried at 150 °C, and weighed. A correction was applied for the solubility of sodium toluene-*p*-sulphonate in acetone (1·2 mg/ml; 1·9 mg/ml for the *p*-nitrobenzenesulphonate). The filtrate was titrated with standard sodium thiosulphate solution. After removal of the acetone by distillation the mixture was diluted with much water and starting material recovered by filtration. The results are shown in the tables. The recovered *trans*-1,2-ditosyloxycyclohexane had m.p. 100–106 °C (after 8 hr), 98–106 °C (16 hr), 102–105 °C (32 hr); the other recovered compounds had substantially unchanged melting points.

(ii) *Camphane Derivatives.* The ditoluene-*p*-sulphonates (0·239 g) were treated as under (i) but were submerged in boiling water. The following results were obtained by the methods described under (i), the figures showing moles of NaOTs and equivs. of iodine: 2-*endo*,3-*exo*-isomer, 0·25 hr, 0·45, 0·08; 0·5 hr, 0·65, 0·10; 1 hr. 0·93, 0·17; 2 hr, 1·39, 0·24; 2-*exo*,3-*exo*-isomer: 4·25 hr, 0·37, 0·09; 8·5 hr, 0·65, 0·18; 17 hr, 0·83, 0·37; 34 hr, 0·97, 0·59; 2-*exo*,3-

endo-isomer: 0·5 hr, 1·06, 0·56. The 2-*endo*,3-*endo*-isomer was recovered in 97% yield after 94 hr at 100 °C. For the di-*p*-nitrobenzenesulphonate of camphane-2-*endo*,3-*exo*-diol (0·2705 g) at 78 °C: 1 hr, 1·58, 0·53.

(*c*) *Isolation of* cis-2-*Iodocyclohexyl Toluene*-p-*sulphonate* (III).—*trans*-1,2-Ditosyloxycyclohexane (3 g), sodium iodide (11·25 g), and anhydrous acetone (75 ml) were heated in a sealed tube at 78 °C for 28 hr. The precipitated sodium toluene-*p*-sulphonate (1·06 g, 39%) was collected by filtration; iodine was titrated (19%), and acetone removed by distillation (the distillate readily decolorized bromine in chloroform). The remaining oil solidified and was crystallized from methanol to give starting material, m.p. 108–109 °C (1·91 g, 63%). The mother liquors were evaporated and the residue crystallized from light petroleum (60–80 °C) to give prisms of the *iodo compound* (0·28 g, 10·5%), m.p. 89·5–90·5 °C. Recrystallization from methanol raised the m.p. to 90–91 °C (Found: C, 41·2; H, 4·5; I, 33·0%. Calc. for $C_{13}H_{17}O_3SI$: C, 41·1; H, 4·5; I, 33·4%).

This compound (0·063 g) was heated with sodium iodide (0·25 g) and anhydrous acetone (1·6 ml) at 78 °C. Results, obtained as under (*b*), were: 3 hr, 0·33, 0·51; and 6 hr, 0·54, 0·94.

IV. Acknowledgments

The authors are indebted to Dr. E. Challen and Mr. D. Weeden for carrying out the microanalyses.

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DIFFUSION ACROSS A LIQUID–LIQUID INTERFACE

By H. Watts*

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Summary

The rate of diffusion of iodine across the liquid–liquid interface between potassium iodide solution and carbon tetrachloride has been investigated. The initial rate of diffusion depends only on the concentration of free iodine in the solution and is not influenced by the iodine combined as I_3^-. There is no initial interfacial resistance to diffusion.

I. Introduction

Whitman and Lewis (1924) suggested that any resistance to interphase diffusion in liquids was due solely to a thin, stagnant film of liquid on each side of the interface and not to the interface itself. This suggestion is known as the "two film theory". However, Tung and Drickamer (1952) found evidence of interfacial resistance to diffusion in the binary liquid system SO_2–n-heptane. If the diffusate is ionized in the phase from which diffusion occurs, and is not ionized in the phase into which diffusion occurs, the rate of diffusion may be influenced by the rate of recombination of the ions. This was observed in the experiments of Lewis (1957) in which uranyl nitrate diffused from water into various organic solvents. Lewis also observed that an interfacial resistance built up during the course of an experiment.

The present paper describes investigations made on a system in which dissociation of an ion might influence the rate of interphase diffusion. The system examined was that of iodine diffusing from aqueous potassium iodide solution, in which it largely exists as the I_3^- ion, into carbon tetrachloride.

II. Experimental

Experiments were performed in 2 cm cells on a Hilger "Spekker". The cells were placed in a water-jacketed cell carrier, constructed in the workshop of this laboratory, through which water from a thermostat was circulated. A thermometer was incorporated in the jacket to measure the cell temperature. CCl_4 (10 ml) was placed in the cell, and I_2 solution (5 ml) in 0·1M aqueous KI was carefully run on top of it. The light beam of the "Spekker" passed entirely through the CCl_4 layer, so that the increase of I_2 concentration in this layer was followed directly by measurement of light absorption. The light absorption of the CCl_4 layer was recorded at 5 min intervals. The cell carrier also held comparator cells filled with a standard solution of I_2 in CCl_4 and pure CCl_4.

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With spectrum violet filters in the light beams, the percentage light absorption by the solution was found to be a linear function of I_2 concentration, within the concentration range used in these experiments.

The solutions were not stirred during an experiment. Mixing of each phase took place solely through convection currents caused by density changes in the solutions due to I_2 diffusing from the upper layer to the lower layer; thus the I_2 concentration in the CCl_4 was not uniform throughout but the light absorption recorded the average I_2 concentration (see Appendix I). Now convection in a liquid must be a faster process than molecular diffusion, since it involves a motion superimposed on diffusion. Thus I_2 will be transported up to the interface faster than it can diffuse through the laminar layers at the interface. The lack of stirring is thus not considered to cause any serious error in the observed rates of diffusion through the interface. Also, only the initial rate of diffusion is considered in interpretation of the results since when the diffusion has proceeded for some time the concentration at the interface may not equal the average concentration of the solution.

Experiments were performed at 15 and 30 °C over a range of initial I_2 concentration from 5 to 26 mM/l in 0·1M aqueous KI. At least four determinations were made at each concentration.

Some experiments were also performed in a 1 cm cell, which might be expected to have different convention characteristics to a 2 cm cell.

"Analar" grade reagents were used without further purification in the preparation of all solutions.

III. Results

Figure 1 shows a typical plot of I_2 concentration in the CCl_4 layer as a function of time; this curve is for an initial concentration of 21·4 mM/l of I_2 in the KI layer at 15 °C. The initial rate of diffusion was obtained from the slope of the tangent at the origin. The mean deviation of four rates at the same concentration from their mean varied between 1% and 4% at different concentrations. The initial diffusion rates are plotted as a function of initial I_2 concentration in Figure 2. The rates are expressed as mM of I_2 transferred/cm^2 of interface/min. Each point represents the mean of at least four results. Results obtained with 1 cm cells agreed with those obtained with 2 cm cells within experimental error.

The free I_2 concentration in the KI solution layer was calculated from the equilibrium constant, K, for the reaction:

$$I^- + I_2 = I_3^-,$$

using the values (concentrations in K are expressed as moles/l),

$$\log_{10} K = 2{\cdot}96 \text{ at } 15 \text{ °C},$$
$$\log_{10} K = 2{\cdot}83 \text{ at } 30 \text{ °C}.$$

These values were obtained from the best line through values of K, given in the literature, plotted against temperature (Bjerrum, Schwarzenbach, and Sillen 1958). The initial rate of diffusion is plotted against free I_2 concentration in Figure 3.

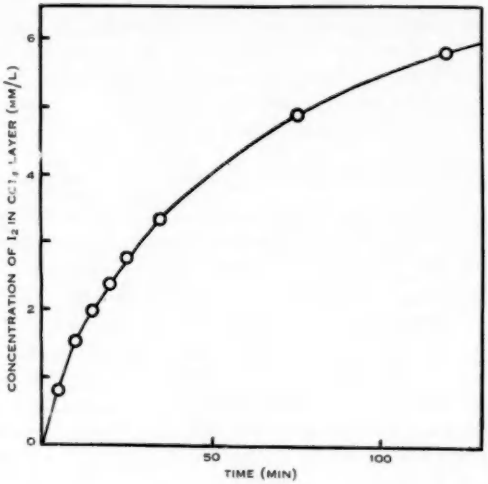

Fig. 1.—Increase of concentration of I_2 in CCl_4 layer for initial concentration 21·4 mM/l I_2 in KI layer.

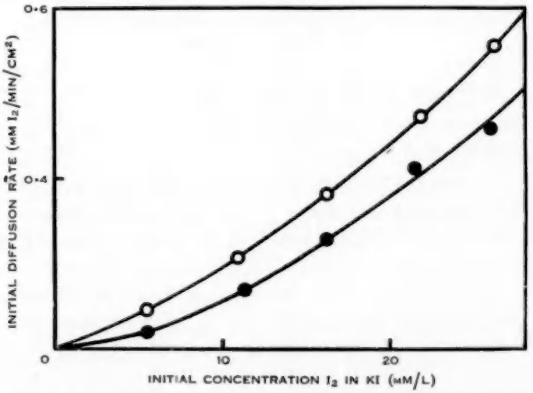

Fig. 2.—Effect of total I_2 concentration on initial diffusion rate.
● 15 °C; ○ 30 °C.

IV. Discussion

It will be seen from Figure 3 that the rate of diffusion is first order with respect to free I_2 concentration over half of the concentration range and only deviates slightly from first order over the remainder of the range. The rate is not first order with respect to total I_2 concentration (Fig. 2), nor is it any simple function of total I_2 concentration. Since diffusion across the interface would be expected to be a first-order process, the rate of dissociation of the I_3^- ion into free I_2 and I^- ion must be much slower than the diffusion process, otherwise the rate would depend on total iodine concentration.

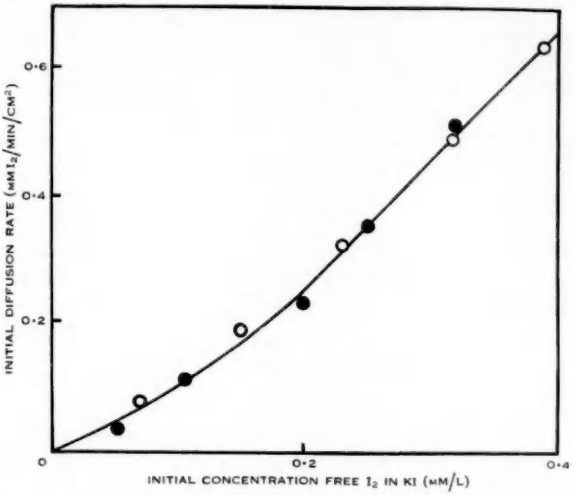

Fig. 3.—Effect of free I_2 concentration on initial diffusion rate.
● 15 °C; ○ 30 °C.

It is seen from Figure 3 that the initial rates at 15 and 30 °C are the same, hence the diffusion has a zero activation energy. Thus the interface offers no resistance to diffusion in this system.

No simple relationship between the variation in rate during an individual experiment and the departure of the system from equilibrium was found. The departure from equilibrium was expressed as $(C_A - DC_B)$, where C_A is the total I_2 concentration in the KI layer, C_B is the concentration in the CCl_4 layer, and D is the distribution coefficient. Absence of a simple relationship between the rate and departure from equilibrium may be due to comparatively slow dissociation of the I_3^- ion or the build up of an interfacial resistance to the diffusion with time, or both.

Experiments of this type might be used to measure the rate of dissociation of an ion if an independent assessment of any possible change of interfacial resistance during an experiment could be made.

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Appendix I

When the concentration is non-uniform the assumption that the light absorption measures the average concentration gives rise to some error. However, it may be shown by a simple numerical treatment that the error is small.

Consider the light path through the solution to be divided into a number of equal volume elements, in each of which the concentration is uniform. In the concentration range studied it was found that the light absorption was a linear function of I_2 concentration. Hence it is a simple matter to compute the total light absorption of a number of volume elements containing different concentrations and compare this with the light absorption of the same number of elements each containing the average concentration. The incident light intensity of each

Table 1

LIGHT ABSORPTION BY SOLUTIONS OF NON-UNIFORM CONCENTRATION

I_2 Concentration in Each Volume Element (arbitrary units)										Total Light Absorption (%)
5	5	5	5	5	5	5	5	5	5	40·1
1	4	1	20	2	3	2	5	2	10	41·4
20	1	2	10	3	4	1	5	2	2	41·4
3	1	2	4	2	2	5	10	1	20	41·2
10	3	5	4	2	1	6	8	4	7	40·4
1	1	1	1	40	1	1	1	1	2	35·7
40	1	1	1	2	1	1	1	1	1	35·7

element is the emergent intensity of the previous element. Table 1 shows the results of calculations for several series of 10 elements with different concentrations and one series containing the average concentration. In this calculation the concentration units are arbitrarily chosen such that one concentration unit in one element absorbs 1% of the light passing through that element. The last column records the total light absorption, and except when the concentration variation is extreme the error is small. Such an extreme concentration variation is unlikely to occur frequently and would be shown by a scatter of points about a smooth curve in the plots of I_2 concentration in the CCl_4 layer as a function of time, of which Figure 1 is typical.

THE THERMAL DECOMPOSITION OF SILVER(II) OXIDE

By J. A. ALLEN*

[*Manuscript received July 6, 1960*]

Summary

The thermal decomposition of silver(II) oxide in a vacuum has been studied over the range 83–134 °C. The rates are quantitatively accounted for by the Polanyi-Wigner equation, the values of the vibration frequency at all stages being within a power of 10 of the expected theoretical value. Changes in the rate at different stages of the reaction are interpreted in terms of diffuseness of the reaction interface. This conclusion is supported by X-ray and surface area studies. The activation energy is 30 kcal.

The activation energy associated with the removal of oxygen from the first layer has been shown to be the same as that for the subsequent reaction.

I. INTRODUCTION

Reactions of the type,

$$A(solid) \rightleftarrows B(solid) + C(gas),$$

have been extensively studied in recent years. Much of the experimental data have been reviewed by Garner (1955) while Jacobs and Tompkins (1955) have sought to unify theoretically the variety of kinetic results that have been reported. Reactions in which B is a metal are often characterized by the ability of the atoms produced to diffuse, aggregate, and crystallize under the conditions of the reaction. An example of this behaviour is afforded by the reaction,

$$2Ag_2O(s) \rightleftarrows 4Ag(s) + O_2(g),$$

which was the subject of a recent paper (Allen 1960). The present work is concerned with the reaction,

$$4AgO(s) \rightleftarrows 2Ag_2O(s) + O_2(g),$$

in which features associated with the mobility of the product B as such may be expected to be absent.

The form of the curves relating the extent of decomposition with time can usually be described in terms of the formation of nuclei of B and their subsequent growth. With certain basic assumptions Jacobs and Tompkins (1955) have shown how kinetic equations of several types may be derived, but have emphasized that *a posteriori* agreement between a theoretically derived rate equation and experimental results does not necessarily confirm the bases on which the former was derived. Further information, e.g. measurements of surface area and the identification of new phases by X-ray analysis, or other means, is of considerable value.

The discrimination possible in the experimental kinetic measurements themselves can often be improved to a degree that places elucidation of the

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reaction mechanism on firmer ground. In the present work, an attempt has been made to improve the discrimination of the kinetics by measuring directly the rate of reaction at its various stages rather than to determine the integral curve relating the extent of the reaction with time. While the latter may be entirely satisfactory in cases where the processes of nucleus formation and growth are readily separable, it will yield much less useful information if the sigmoidal curve relating the extent of reaction with time departs to a small degree only from a straight line.

II. Thermodynamics and Structure

The equilibrium pressure of oxygen for the reaction,

$$4AgO(s) \rightleftarrows 2Ag_2O(s) + O_2 \qquad (1)$$

has not apparently been determined directly. In the absence of experimental thermodynamic data for AgO an estimate may be made by comparison of the values for CuO and Cu_2O with the structurally similar AgO and Ag_2O. The appropriate data given by Kubaschewski and Evans (1958) and McMillan (1954) are listed in Table 1. Later structural data for AgO by Scatturin, Bellon, and Zannetti (1958) differ slightly from those recorded in the table.

Table 1

THERMODYNAMIC AND STRUCTURAL DATA FOR COPPER AND SILVER OXIDES

Oxide	$-\Delta H_{298}$ (kcal/g-atom metal)	S_{298} (cal/deg/ g-atom metal)	Structure	Lattice Constants	Groups per Unit Cell
CuO	37·1	10·2	Monoclinic	4·65 ; 3·41 ; 5·11 ; 99° 29′	4
Cu_2O	20·0	11·22	Cubic	4·25	2
AgO*	(6·8)	(13·2)	Monoclinic	5·79 ; 3·50 ; 5·51 ; 107° 30′	4
Ag_2O	3·65	14·55	Cubic	4·72	2

* The values in parentheses for AgO are estimated by direct proportion.

For reaction (1): $\Delta H_{298}=12{\cdot}6$ kcal and $\Delta S_{298}=54{\cdot}4$, whence

$$\log_{10} p_{atm} = -\frac{2740}{T} + 11{\cdot}8. \qquad (2)$$

The estimated dissociation pressures at 50 and 150 °C are therefore, respectively, $10^{3{\cdot}3}$ and $10^{5{\cdot}3}$ atm; for subatmospheric pressures the reverse reaction may therefore be wholly neglected. The assumption that ΔH does not vary appreciably with temperature does not affect this conclusion.

The structural data for AgO enable an estimate to be made of the average area per atom of oxygen on a surface of the oxide. The volume per atom of oxygen calculated from the lattice constants is 26·6 Å^3. The area per oxygen atom is taken at $(26{\cdot}6)^{2/3}=8{\cdot}9$ Å^2.

The volume ratio of Ag_2O to AgO per atom of silver calculated from the lattice constants given in Table 1 is 0·945. On this basis a reaction product

conforming to the lattice of Ag_2O will occupy a somewhat smaller volume than the AgO from which it came. It is to be noted, however, that the actual volume ratio may differ to some extent from that calculated from the X-ray data because of the presence of structural defects in the reactant and product.

III. Experimental

(*a*) *Preparation of Silver Oxide*

The oxide was prepared according to the directions of Hammer and Kleinberg (1953). After drying in air at 80 °C overnight the cake was lightly ground and stored over silica gel. The specific surface area determined by nitrogen adsorption was 126×10^2 cm^2/g. No attempt has yet been made following the work of de Boer and van Ormondt (1952) to examine the effect of the incorporation of foreign metal atoms on the stability of the oxide.

(*b*) *Apparatus*

The main apparatus was a standard gas adsorption unit differing only in minor detail from that described by Harkins and Jura (1944). The volumes of the connecting tubes were determined by calibration with helium. Gas adsorption surface areas were measured with nitrogen purified over evaporated sodium. Decomposition experiments were made in the same apparatus.

The X-ray studies were carried out using a Philips unit type PW1009. Samples were mounted in Lindemann tubes in a powder camera (Philips type number 33524) of diameter 114·83 mm. An X-ray tube with a copper target operated at 40 kV and 20 mA was employed throughout.

(*c*) *Procedure*

(i) *Decomposition in a Vacuum.*—Samples of oxide (1 g) at constant temperature were evacuated continuously. At suitable time intervals the measuring apparatus was isolated from the pumps and the increase in pressure with time in a system of constant volume and temperature measured. For a given short time interval, dV/dt, the rate of evolution of gas thus measured was constant. Measurements during a series of such intervals interspersed with periods of evacuation yield values of dV/dt as a function of time. These functions were established for five temperatures between 83·5 and 134·3 °C.

To fix a zero of time, the heating was carried out from room temperature over periods all between 10 and 15 min. The zero of time was taken as the time at which the apparatus was first isolated from the pumps at the end of the heating period. Experiments designed specially for the purpose showed that the volume of gas evolved during the heating period could be computed satisfactorily from ($\frac{1}{2} \times$rate of evolution at cut-off $\times$time of heating). The correction is negligibly small except in relation to the initial desorption of gas from the surface.

(ii) *Surface Area Measurements.*—It was shown experimentally that for a particular temperature the decomposition could be interrupted by removing the heating furnace and subsequently resumed without affecting the course of the decomposition. Adjustment for the cooling and heating periods brought the rates into line with those before interruption. Surface areas were measured in this way on the same sample at several stages of the decomposition. The

standard B.E.T. procedure was employed using at least five points on the isotherm. The area per molecule of nitrogen used in calculating the surface area was 16·2 Å².

(iii) *X-Ray Powder Photographs.*—The specimens used were decomposed to a desired extent at a particular temperature, rapidly cooled, and examined at room temperature. The powder photographs obtained after an exposure time of 2 hr were processed in a uniform manner and compared with patterns of AgO and Ag_2O obtained singly under identical conditions.

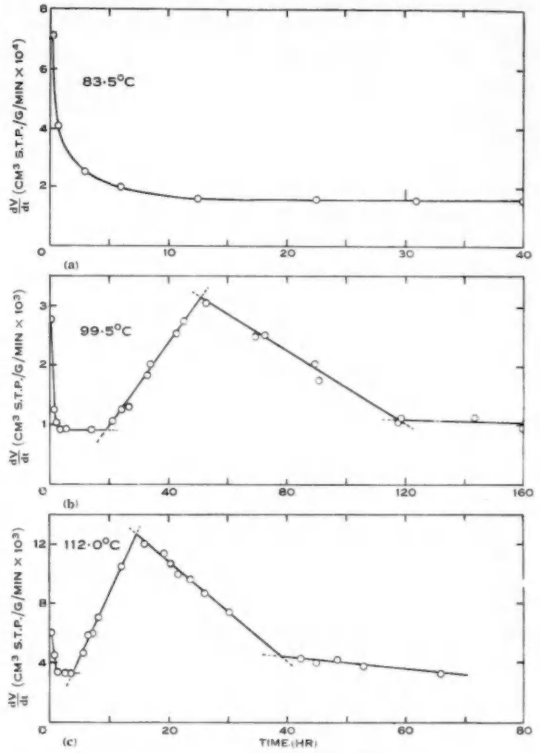

Fig. 1.—Rates of decomposition of silver(II) oxide. (*a*) 83·5 °C; (*b*) 112·0 °C; (*c*) 124·0 °C.

IV. Results

(*a*) *Kinetic Results*

The kinetic results are summarized in Figures 1 (*a*), 1 (*b*), and 1 (*c*), together with Figures 2 (*a*) and 2 (*b*), for the five temperatures 83·5, 99·5, 112·0, 124·0, and 134·3 °C. It may be noted that the rate and time scales have been changed in each case in order to show the results as a whole. Figure 2 (*c*) shows schematically the general type of behaviour. Five stages may be distinguished,

as follows: (1) An initial "desorption", blending into (2) an initial constant rate, followed by (3) a rate increasing linearly with time to a maximum, (4) a rate decreasing linearly with time to a break point (5) a rate continuing to decrease more or less linearly with time, but at a slower rate.

Figure 1 (*a*) shows only stages 1 and 2 because the experiment was not carried on for a sufficient length of time. The break point between 4 and 5 is not shown in Figure 2 (*a*) because of the omission of the latter part of the

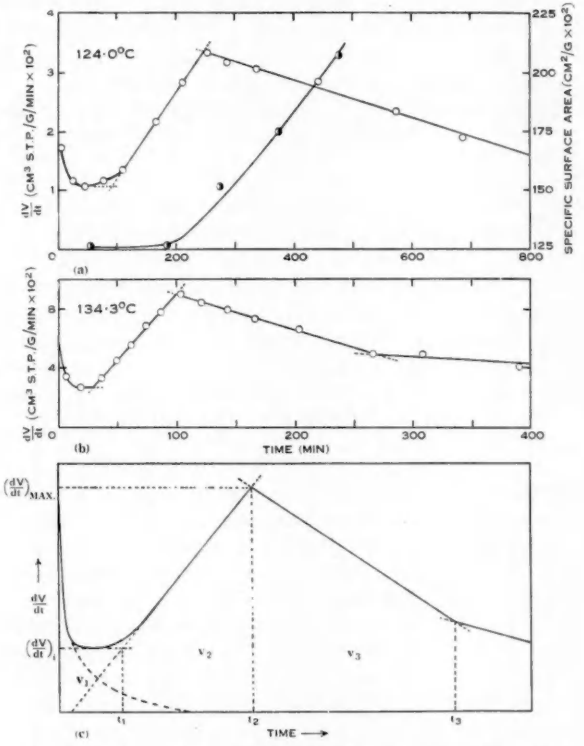

Fig. 2.—Rates of decomposition of silver(II) oxide and specific surface area during decomposition. (*a*) 124·0 °C; (*b*) 134·3 °C; (*c*) schematic representation of decomposition.

curve. The main divisions are distinguished (Fig. 2 (*c*)) by the times t_1, t_2, and t_3 and the corresponding volumes by v_1, v_2, and v_3. The overall behaviour is shown in the conventional curves for degree of decomposition against time in Figure 3.

(1) *Initial Desorption.*—Stages 1 and 2 may be considered in two ways. Stage 2 may be considered to arise from a combination of a desorption curve approaching zero rate and a rate (stage 3) increasing linearly from zero. This

possibility is shown by broken lines in Figure 2 (*c*). Alternatively, the constant initial rate (stage 2) may be considered to commence at zero time, and to be obscured by the desorption of gas taking place from the free surface at an enhanced rate until a particular surface coverage depending on the temperature is achieved.

Detailed examination of the experimental curves shows that the first possibility does not fit quantitatively ; in Figure 1 (*a*) in particular the constant rate observed over at least 30 hr could not be accounted for in terms of the first possibility. Examination of the experimental data in terms of the second alternative has therefore been preferred.

The volume of gas desorbed in the first stage until the constant rate is observed experimentally may be obtained by graphical integration, the necessary correction being applied for the gas evolved during the heating period as outlined in Section III. A knowledge of the volume of gas corresponding to maximum occupation of the surface enables the volume remaining adsorbed at the pressure in the apparatus at the several temperatures to be obtained by difference. The monolayer capacity, estimated from the measured specific surface area of 126×10^2 cm^2/g and an area per oxygen atom of 8·9 Å^2, is 0·26 cm^3 at S.T.P. The resulting isobar is shown in Figure 4, from which it is evident that the initial desorption (stage 1) involves only the first oxygen layer. Extrapolation of Figure 4 to lower temperatures shows that the layer is virtually complete at about room temperature and effectively zero at temperatures above 134 °C.

An estimate of the heat of adsorption may be obtained in the following way, based in part on the presentation by Trapnell (1953). The velocities of adsorption, u, and desorption, u', on a uniform surface are given by the equation

$$u = \frac{\sigma p}{\sqrt{(2\pi mkT)}} f(\theta) e^{-E/RT}, \tag{3}$$

$$u' = k' f'(\theta) e^{-E'/RT}, \tag{4}$$

where σ is the condensation coefficient, p the pressure, m the mass of the gas molecule, E and E' the activation energies of adsorption and desorption, respectively, and $f(\theta)$ and $f'(\theta)$ functions of the coverage θ ; k and k' are constants. At equilibrium $u = u'$ and since $E' - E = q$, the heat of adsorption,

$$p = \frac{k'}{\sigma} \sqrt{(2\pi mkT)} \frac{f'(\theta)}{f(\theta)} e^{-q/RT}. \tag{5}$$

Over a small temperature range the change in $\sqrt{(2\pi mkT)}/\sigma$ will be small in relation to $\exp(-q/RT)$ for expected values of q and we may write

$$p \approx K \frac{f'(\theta)}{f(\theta)} e^{-q/RT}, \tag{6}$$

where K is a constant.

The simplest forms of $f(\theta)$ and $f'(\theta)$ are given by

$$f(\theta) = 1 - \theta, \tag{7a}$$

$$f'(\theta) = \theta, \tag{7b}$$

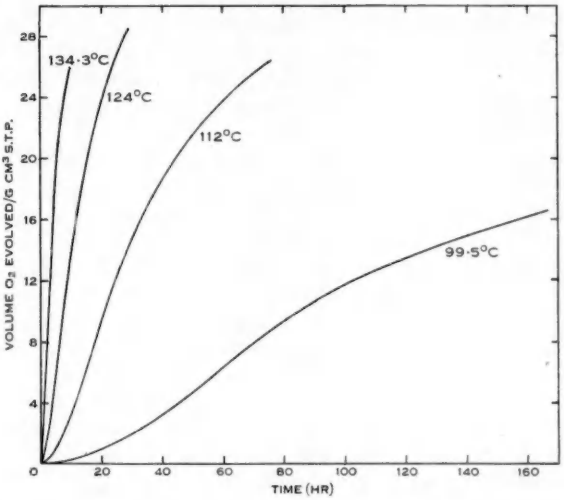

Fig. 3.—Integral curves for degree of decomposition against time.

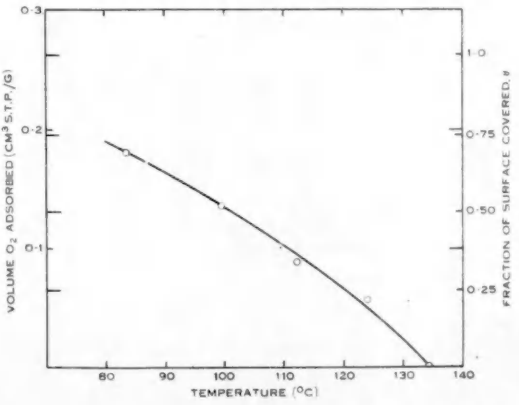

Fig. 4.—Adsorption isobar of oxygen on silver(II) oxide.

for mobile and immobile layers involving the occupation of each site by a molecule, and

$$f(\theta)=(1-\theta)^2, \tag{8a}$$

$$f'(\theta)=\theta^2, \tag{8b}$$

for mobile layers involving one atom per site. Both cases may be included in

$$p\approx K\left(\frac{\theta}{1-\theta}\right)^n e^{-q/RT}, \tag{9}$$

where n is 1 or 2.

From the series of values of θ and hence $\theta/(1-\theta)$ for a succession of values of T at a constant value of p, the plot of $\log_{10}[(1-\theta)/\theta]$ against $1/T$ should yield a straight line of slope $-q/2{\cdot}303nR$. The results in this form are shown in Figure 5, from which $q=13{\cdot}9n$ kcal.

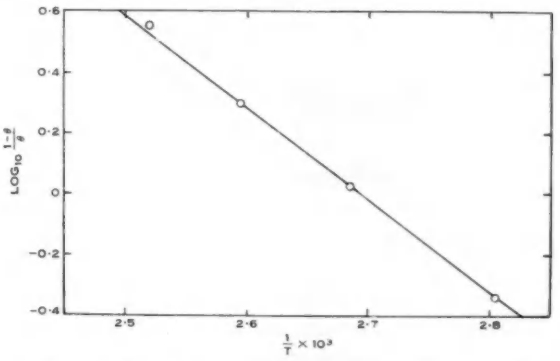

Fig. 5.—Plot of $\log_{10}[(1-\theta)/\theta]$ against $1/T$ for determination of heat of adsorption of oxygen on silver(II) oxide.

It is also of interest to make an estimate of the activation energy of desorption. Since the rates of desorption as functions of time have been determined experimentally, these rates as functions of the volume of gas desorbed may be obtained by graphical integration. In Figure 6 the logarithms of the rates for various values of the volume desorbed are plotted as a function of the reciprocal of the temperature; the activation energies so obtained lie between 26 and 30 kcal. It is obvious that these results are of low accuracy and no good purpose is served in trying to examine the possible variation of the activation energy with coverage.

The value of 26–30 kcal for the energy of activation for desorption may be compared with the value for the heat of adsorption of $13{\cdot}9n$ kcal, where $n=1$ or 2. While the possibility that $n=1$ cannot be ruled out it would appear more likely that $n=2$ and E (eqn. (3)) is zero.

(2) *The Initial Constant Rate* (*0 to* t_1).—The logarithms of the initial constant rates are plotted against $1/T$ in Figure 7. The fit is good and the activation energy calculated from the slope is 29·7 kcal. The volumes v_1 (see Fig. 2 (*c*))

calculated from the initial constant rate from $t=0$ to $t=t_1$ for the several temperatures are included in Table 2.

The reasonable constancy of v_1 of 0·9 cm³ at S.T.P/g shows that at 83·5 °C the decomposition would have to be continued for about 100 hr before the rate would begin to increase. The average value of v_1 corresponds to 2% decomposition.

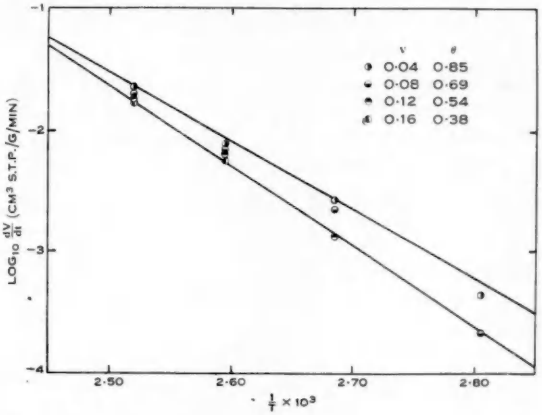

Fig. 6.—Plot of log $_{10}$ (rate of desorption) against $1/T$ for determination of activation energy of desorption of oxygen from silver(II) oxide.

(3) *Rate Increasing Linearly to a Maximum* (t_1 *to* t_2).—The logarithms of the maximum rates are plotted against $1/T$ in Figure 7. The activation energy calculated for the slope is 29·2 kcal. The total volume of oxygen evolved to

TABLE 2

VOLUMES OF OXYGEN (CM³ S.T.P/G) AT VARIOUS STAGES IN THE REACTION

Temp. (°C)	v_1	v_2	v_3	Σv
83·5	—	—	—	—
99·5	1·0	3·9	8·5	13·4
112·0	0·8	5·0	12·6	18·4
124·0	0·95	3·5	14·1	18·55
134·3	0·8	4·1	12·0	16·9

reach the maximum is v_1+v_2 and from Table 2 is fairly constant, at 5·0 cm³ S.T.P. corresponding to decomposition to the extent of 11%. Between t_1 and t_2 the rate may be represented by

$$\frac{dV}{dt}=\left(\frac{dV}{dt}\right)_i+k_1(t-t_1), \qquad (10)$$

where $(dV/dt)_i$ is the constant initial rate and k_1 is a con tant. The plot of $\log k_1$, that is, $\log d^2V/dt^2$, against $1/T$ is included in Figure 7, the calculated activation energy associated with k_1 being 58 kcal.

(4) *Rate Decreasing Linearly to a Break Point* (t_2 *to* t_3).—In this region the rate may be expressed by the equation

$$\frac{dV}{dt} = \left(\frac{dV}{dt}\right)_{max.} - k_2(t - t_2) \qquad (11)$$

where $(dV/dt)_{max.}$ is the maximum rate. The plot of $\log k_2$, that is, $\log (d^2V/dt^2)$ against $1/T$ is included in Figure 7, the associated activation energy being 52·3 kcal.

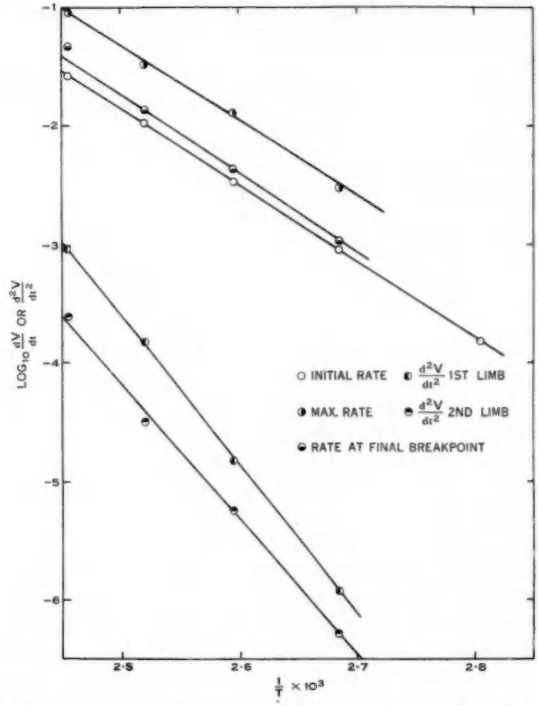

Fig. 7.—Arrhenius plots for rates of decomposition of silver(II) oxide.

The volumes evolved to the break point at t_3 are given in Table 2, namely, $v_1+v_2+v_3$, averaging 16·8 cm³ at S.T.P. and corresponding to an extent of decomposition of about 37%.

The logarithms of the rates at the break points plotted against $1/T$ are included in Figure 7, the activation energy calculated from the slope being 31 kcal.

(5) *The Decay Period.*—The decay period covering stages (4) and (5) has been examined in terms of the Avrami-Erofeyev equation and the unimolecular decay law (Jacobs and Tompkins 1955). These equations, respectively, take the forms,

$$\alpha = 1 - e^{-kt^3}, \tag{12}$$

and

$$\ln \frac{1}{1-\alpha} = kt, \tag{13}$$

where α is the degree of decomposition. Equation (12) does not describe the experimental data, but the data may be expressed satisfactorily in terms of (13) with different values of k (k_3 and k_4) for the separate stages (4) and (5), respectively. The results are summarized in Table 3.

TABLE 3

APPLICATION OF UNIMOLECULAR DECAY LAW TO STAGES (4) AND (5)

Temp. (°C)	k_3 ($hr^{-1} \times 10^2$)	k_4 ($hr^{-1} \times 10^2$)
99·5	3·16	2·10
112·0	16·7	9·02
124·0	41·8	30·7
134·3	143·5	82·3

The activation energy associated with k_3 is 33·3 kcal and with k_4, 31·8 kcal.

(*b*) *Surface Area Results*

Measurements of the change in surface area were confined to the decomposition at 124 °C. The main results are plotted in Figure 2 (*a*).

(*c*) *X-Ray Results*

Measurements were confined to decomposition at 124 °C. The comparative analysis of the powder patterns showed that the Ag_2O lattice detected primarily by the two strongest reflections (111 and 200) begins to appear just before the maximum rate is reached and is unmistakeable at the maximum and thereafter. The results are summarized in Table 4. Comparison of the rates listed with Figure 2 (*a*) indicates the points at which the samples were taken.

V. DISCUSSION

The constancy of the activation energies determined at the three characteristic points at t_1, t_2, and t_3 and the constant values obtained for the extent of decomposition of these points, suggest that the regular increase in rate between t_1 and t_2 and the decrease between t_2 and t_3 are likely to be associated with changes in the reaction geometry. Following the suggestion of Bradley (1931) and Topley (1932), we may examine this hypothesis in terms of the Polanyi-

Wigner equation. The rapid nucleation as the first step in the decomposition evident from Figure 3 also points to the use of this equation which takes the form,

$$-\frac{dN}{dt}=\nu\tilde{N}Ae^{-E/RT}, \tag{14}$$

where $-dN/dt$ is the rate of removal of molecules from the interface, $\tilde{N}$ is the number of molecules per unit area, A the area of the interface, and E the activation energy. ν is the vibration frequency which theoretically should have a value of 5×10^{12}/sec.

TABLE 4

X-RAY IDENTIFICATION OF OXIDE PHASES
Temperature, 124 °C

Sample No.	Rate Attained ($cm^3/g/min$)	Pattern	Remarks
0	0	AgO	Untreated sample
10	$2\cdot0\times10^{-2}$	AgO	Before maximum
8	$3\cdot0\times10^{-2}$	AgO+trace Ag_2O	Before maximum
9	$3\cdot4\times10^{-2}$	AgO+Ag_2O	Maximum rate
7	$2\cdot7\times10^{-2}$	AgO+Ag_2O	Beyond maximum rate
4	$8\cdot4\times10^{-3}$	Ag_2O	After 29 hr decomposition

If this equation is employed in conjunction with the constant initial rates measured experimentally, E is known at 29·7 kcal, A may be taken as the geometric area, namely, 126×10^2 cm^2/g and $\tilde{N}$ calculated on the basis that the area occupied per " molecule " at the interface is 17·8 $Å^2$, that is, twice the area per oxygen atom. $-dN/dt$ is known from the measured values of the rate of evolution. For the five temperatures studied the values of ν range from 1·8 to $1\cdot5\times10^{13}$ with an average value of $1\cdot6\times10^{13}$. The agreement with the theoretical value is good, one or two powers of 10 usually being considered satisfactory agreement. If applied to the maximum rate $\nu\tilde{N}A$ would have to be about 3·5 times larger and to the rates at the break point approximately the same as for the constant initial rates. At any point over about the first 40% of the reaction studied the Polanyi-Wigner equation correctly predicts within a power of 10 the reaction rate.

Since v_1 would involve only a very few layers at the surface, the reaction interface would up to this point remain sensibly constant in area. Beyond v_1 the interfacial area would in the absence of the crystallization of a new phase be expected to become progressively more diffuse, thereby increasing A. An increase by a factor of about 3·5 does not appear unreasonable.

At the maximum rate (t_2) it would on this basis be necessary to suppose that the interface starts to become less diffuse. Such a situation could arise if a new phase of distinctive crystal form began to appear in substantial quantities. That this is so is clearly shown by the X-ray data (Table 4), though it should be

noted that Ag_2O crystals too small in size or of insufficient quantity to diffract could be present before the X-ray pattern is detectable.

A comparison of the X-ray data and the results of the surface area measurements shows that the total surface area begins to rise at about the point at which the Ag_2O phase begins to appear in the X-ray patterns. If the Ag_2O is less voluminous per silver atom than the AgO from which it was derived as predicted by the crystal structures (Section II), it is obvious that either tension cracks must in due course appear, or the Ag_2O is composed of crystals smaller than the AgO parent. In either case the increase in surface area is readily explicable. Furthermore, the applicability of the unimolecular decay law and not the Avrami-Erofeyev equation means that there must be a collapse of the interface.

The analysis of stage 1 of the reaction has been formally on the basis of a surface desorption. Though the data in this region are less accurate than those in the later stages, the most probable explanation is that this stage involves the removal of oxygen from a mobile atomic layer which at most does not involve oxygen atoms more than one or possibly two atomic distances below the free surface. The similarity in activation energy for this stage and that for the reaction rates at later stages and its equality with the heat of adsorption are conditions which serve to confirm the applicability of the Polanyi-Wigner equation. In physical terms this means rapid nucleation over the whole surface followed by a rate governed by the progression of the interface towards the centre of the crystal.

VI. Conclusions

It may be concluded that the kinetics of the thermal decomposition of AgO to Ag_2O and oxygen in the range 83·5 to 134·3 °C are quantitatively accounted for by the Polanyi-Wigner equation. Changes in rate at different stages of the reaction are interpreted in terms of the diffuseness of the reaction interface, a view supported by surface area and X-ray data. The activation energy is 30 kcal.

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SOME STUDIES IN INORGANIC COMPLEXES

IX. THE PYROLYSIS OF HEXAMMINEOSMIUM(III) SALTS

By G. J. SUTTON*

[*Manuscript received July* 28, 1960]

Summary

A study has been made of the action of heat on the salts $Os(NH_3)_6ISO_4$ and $Os(NH_3)_6X_2$, in which X is bromide or iodide. The initial products are $[OsI(NH_3)_5]SO_4$ and $[OsX(NH_3)_5]X_2$ respectively. At higher temperatures the latter decompose into black double-halogen bridged complexes $[(NH_3)_5XOsX_2OsX(NH_3)_2]X_2$, while the sulphate forms a black residue of composition corresponding to $Os(NH_3)_2ISO_4$ but of unknown structure. Further heating results in oxidation to OsO_4 and halogen in all cases. The structures of the black halogen complexes as well as the monohalogenopentammineosmium(III) salts have been verified by magnetic and conductance measurements. However, since osmium(IV) shows a remarkable tendency towards maximum electron pairing, having a moment of 1·2–1·5 Bohr magnetons in most of its complexes, the black compounds could have the less symmetrical structure $[Os(III)X(NH_2)_5][Os(IV)X_5NH_2]$.

I. INTRODUCTION

Dwyer and Hogarth (1950, 1951) and later Watt and Vaska (1958*a*, 1958*b*, 1958*c*) studied the formation of hexammine and monoacidopentammine complexes of osmium(III) by the ammonation of ammonium hexahalido-osmate(IV). Nitrilo-bridged cation complexes were also described. This work was prompted by the results of these investigations.

II. RESULTS

Repetitive investigations have shown that hexammineosmium(III) iodide sulphate loses a molecule of ammonia at 230 °C with the formation of light brown iodopentammineosmium(III) sulphate. Similarly, hexammineosmium(III) iodide and bromide yield brown iodopentammineosmium(III) iodide and light fawn bromopentammineosmium(III) bromide at 160 and 233 °C respectively. Conductance measurements at low concentration in methanol agree with the assumption that they contain monoacido double-charged cations. They are almost insoluble in common solvents excepting the bromo complex, which is appreciably soluble in water, but conductance measurements in this solvent at low concentration are unreliable due to lability of the coordinated halogen. At 234 and 305 °C respectively the monoacido iodide and bromide decompose to black crystalline substances of empirical formula $Os(NH_3)_3X_3$, in which X is halogen. At higher temperatures the latter are oxidized to products which include osmium tetroxide and halogen. Since the magnetic moment of osmium(III) in ammine complexes varies from 1·6 to 2·1 B.M., according to Watt and Vaska (1958*a*, 1958*b*, 1958*c*),

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and that for the osmium(IV) atom is usually from 1·2 to 1·5 B.M., according to various workers including Earnshaw *et al.* (1957), the observed magnetic moment of about 1·7 B.M. in the black complexes is not diagnostic of oxidation state. However, it does indicate that as electrolytes the osmium atoms in cation and anion could have respective formal valencies of either 3 and 3 or 3 and 4. Conductance measurements in methanol show that the black complexes are bi-bivalent electrolytes, which eliminates the structure $[Os(NH_3)_6][OsX_6]$. Furthermore, the latter bromo compound is amber coloured, not black. The most likely structure is (i) dihalidohexammine-μ-dihalidodiosmium(III) halide $[(NH_3)_3XOsX_2OsX(NH_3)_3]X_2$, in which there is a double-halogen bridge between the osmium atoms. A less likely structure is (ii) halidopentammineosmium(III) aminopentahalido-osmate(IV) $[(NH_3)_5XOs(III)][Os(IV)NH_2X_5]$, the formation

TABLE 1

MOLECULAR CONDUCTIVITIES OF OSMIUM(III) COMPLEXES IN 3×10^{-4}M CONCENTRATION IN METHANOL AT 25 °C

Substance	Conductivity (Ω^{-1})	Substance	Conductivity (Ω^{-1})
$[Os(NH_3)_5I]I_2$ ..	180	$[Os_2(NH_3)_6I_4]I_2$ ..	194
$[Os(NH_3)_5Br]Br_2$ ..	183	$[Os_2(NH_3)_6Br_4]Br_2$	182
$[Os(NH_3)_5I]SO_4$ ..	177		

of which would require the removal of a hydrogen atom from an ammonia molecule by atmospheric oxidation. It is not necessary to assume that the black colour indicates the presence of two oxidation states of osmium, since dark colours are quite common in bridged compounds, particularly those containing iodine. The available analytical data are in agreement with either structure, since hydrogen analyses are difficult to obtain in the presence of osmium. The possibility of nitrilo-bridging is discounted, since this would raise the oxidation state of the osmium. Unfortunately, the black substances were found to be too insoluble for ion exchange with triphenylmethylarsonium or periodate ions, which could lead to easier identification. However, it is noteworthy that their absence amongst the original products of ammonation is in accordance with their lack of reactivity

TABLE 2

MAGNETIC MEASUREMENTS CORRECTED TO 20 °C*

Substance	$\chi_g\times10^{-6}$	$\chi_M\times10^{-6}$	$\chi_{M_1}\times10^{-6}$	μ (B.M.)
$[Os(NH_3)_5I]SO_4$	2·8	1395	1532	1·9
$[Os(NH_3)_5Br]Br_2$	2·6	1385	1458	1·8
$[Os_2(NH_3)_6I_4]I_2$	1·7	2120	2434	1·7
$[Os_2(NH_3)_6]Br_2$	1·9	1830	2112	1·6

* 0·08 to 0·11 g substance in a thin tube.

towards ammonia when heated in a pressure vessel. Their conductances and those of the monoacidopentammine complexes with methanol as solvent are summarized in Table 1, whilst measurements of magnetic susceptibility are given in Table 2. Pyrolysis of iodopentammineosmium(III) sulphate at a temperature of 305 °C resulted in the formation of black crystals of a substance of composition corresponding to $Os(NH_3)_3ISO_4$. This could be a mixture of the iodo bridge complex $[Os_2(NH_3)_6I_4]I_2$ and a sulphato complex of osmium.

III. Experimental

The conductance measurements were determined in pure methanol at 25 °C using a Philoscope model GM4249/01 and cell GM4221. The methanol was purified according to the method outlined in Part V of this series (Sutton 1959). Magnetic measurements were carried out by the Gouy method. Osmium was estimated by the colorimetric method of Dwyer and Gibson (1951).

(*a*) *Hexammineosmium(III) Iodide Sulphate.*—The method of Dwyer and Hogarth (1950, 1951) was repeated. Osmium tetroxide (2 g) was treated with concentrated HBr and after 2 days heated to 70 °C and a saturated solution of ammonium bromide added to precipitate black crystals of ammonium hexabromo-osmate(IV). The salt in small portions (2 g) was transferred to a glass tube within an autoclave and subjected to a pressure of 90–110 lb of ammonia for 30 min at room temperature. The pressure was reduced to 40 lb and the autoclave heated to 300 °C for 1 hr. After cooling, excess ammonia was allowed to evaporate and the greyish green residue was treated thrice with water (25 ml). The aqueous extracts were treated with solid KI to a faint precipitate and the filtrate treated with ammonium sulphate (5 g). The ivory-white precipitate of the iodide sulphate was filtered off, washed with NaI solution, ethanol, and ether, and dried (mean yields 0·1 g).

(*b*) *Hexammineosmium(III) Iodide.*—The iodide sulphate was dissolved in the smallest quantity of water at 80 °C and BaI_2 added to a slight excess. The cooled filtrate was treated with NaI in excess and the yellow iodide filtered, washed with ethanol and ether, and dried.

(*c*) *Hexammineosmium(III) Bromide.*—The iodide $Os(NH_3)_6I_3$ was dissolved in a little water and treated with a cold solution of the calculated proportion of $AgNO_3$. The filtrate was treated with excess LiBr and the pale fawn prisms which formed were filtered, washed, and dried as before.

(*d*) *Iodopentammineosmium(III) Sulphate.*—Hexammineosmium(III) iodide sulphate (0·036, 0·037, 0·052 g) was heated at 230 °C in a small electric muffle furnace for 1 hr with the resulting formation of light brown dodecahedra (yields 0·033, 0·033, 0·050 g). The salt was found to be almost insoluble in common solvents, excepting methanol and water, in which it is very sparingly soluble, forming pale orange solutions. It is decomposed by strong alkalis and concentrated strong acids (Found: I, 25·4; SO_4, 19·0%. Calc. for $Os(NH_3)_5ISO_4$: I, 25·5; SO_4, 19·3%). At 305 °C an additional preparation of the salt changed to black crystals with loss of weight corresponding to two molecules of ammonia (Found: I, 27·9; SO_4, 20·9%. Calc. for $Os(NH_3)_3ISO_4$ polymer: I, 27·4; SO_4, 20·7%). At 350 °C the black substance decomposed in air with the evolution of OsO_4 and I_2.

(*e*) *Iodopentammineosmium(III) Iodide.*—Hexammineosmium(III) iodide (0·193 g) was heated at 160 °C for 1 hr, when chocolate-brown dodecahedra resulted (yield 0·186 g) (Found: I, 57·4; Os, 28·9%. Calc. for $Os(NH_3)_5I_3$: I, 58·0; Os, 28·6%). The salt was found to be very sparingly soluble in methanol and water, and insoluble in other common solvents. On heating a second product to 234 °C lustrous black octahedra were found (Found: I, 60·8; Os, 30·5%. Calc. for $Os(NH_3)_3I_3$ polymer: I, 61·3; Os, 30·6%). The substance was completely oxidized at about 320 °C.

(*f*) *Bromopentammineosmium(III) Bromide.*—Hexammineosmium(III) bromide (0·103 g at 266 °C decomposed to light fawn prisms of bromopentammineosmium(III) bromide (0·095 g) (Found: Br, 46·2; Os, 37·0%. Calc. for $Os(NH_3)_5Br_3$ polymer: Br, 46·6; Os, 36·9%). The salt was found to be appreciably soluble in water, sparingly soluble in methanol, ethanol, and

acetone, and insoluble in other solvents. A further preparation was heated to 305 °C, when black crystals were formed (Found : Br, 49·3 ; Os, 39·9%. Calc. for $Os(NH_3)_3Br_3$ polymer Br, 49·7 ; Os, 39·6%). Above 320 °C the substance was oxidized to OsO_4 and other products.

IV. Acknowledgments

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SOME STUDIES IN INORGANIC COMPLEXES

X. CADMIUM AND INDIUM WITH 2-PICOLYLAMINE

By G. J. SUTTON*

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Summary

Colourless or very pale yellow complexes of the type $Cdpic_2X_2$, $Cdpic_3(ClO_4)_2$, $Inpic_2X_3$, and $Inpic_3X_3$ have been prepared and studied, pic being the ligand 2-picolylamine and X halogen. The cadmium complexes are stable in water, in which they may be prepared and in which they react as bi-univalent electrolytes, whereas the iodide $Cdpic_2I_2$, having a very low conductance in nitromethane, has a 6-coordinate octahedral structure. The indium complexes $Inpic_3X_3$ may be prepared in ethanol, whilst the $Inpic_2X_3$ complexes are obtained by pyrolysis of the latter. Conductance measurements of the iodides $Inpic_3I_3$ and $Inpic_2I_3$, as well as those of ethylenediamine $Inen_3I_3$ and $Inen_2I_3$, in nitromethane show that in all cases a 6-coordinate structure is maintained. All the indium picolylamine complexes hydrolyse in water.

I. INTRODUCTION

The formation of 2-picolylamine complexes of the transition metals nickel, copper, and cobalt, as described by Sutton (1960*a*, 1960*b*), prompted the investigation with this ligand and the non-transition metals cadmium and indium. Colourless cadmium complexes of both 1,10-phenanthroline and 2,2′-dipyridyl have been investigated by many workers, as reviewed by Brandt, Dwyer, and Gyarfas (1954). These may contain 1, 2, or 3 molecules of base and appear to be 4- or 6-coordinate. The existence of 6-coordinate octahedral complex formation in trisdipyridyl and trisphenanthrolinecadmium ions has been demonstrated by Douglas, Laitinen, and Bailar (1950). Bisethylenediamineindium halides were prepared and studied by Sutton (1948), and although conductance measurements in ethanol indicated metal-halogen bonding, the results, as well as those with the trisethylenediamineindium complexes, are unreliable owing to the possibility of decomposition in this solvent. A study of water-stable trisdipyridyl and trisphenanthroline complexes of indium was made by Sutton (1949). However, the corresponding bisdipyridyl and bisphenanthroline complexes were not isolated. Accordingly, it was decided to attempt the preparation of both cadmium and indium complexes of 2-picolylamine, in order to further the investigations of 4- or 6-coordinate complex formation by conductance measurements in suitable solvents such as nitromethane and nitrobenzene, in which decomposition is unlikely. It was also decided to carry out similar measurements on tris- and bisethylenediamine indium complexes.

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II. Results and Discussion

When dilute aqueous solutions of cadmium halides are treated with a slight excess of 2-picolylamine, colourless crystals of complexes of the type $Cdpic_2X_2$ are formed on standing, in which pic is 2-picolylamine and X is halogen. Conductance measurements in water show that the substances behave as bi-univalent electrolytes at 10^{-2}M concentration, with some decomposition on further dilution to 10^{-3}M concentration, with the exception of the iodide which is insufficiently soluble for measurements to be made at the higher concentration and appears to be solution-stable at the lower concentration. It is likely that 6-covalency is maintained in dilute solutions by attachment of solvent molecules. None of the complexes is sufficiently soluble in nitrobenzene for conductance measure-

Table 1

Molecular conductances in water at 25 °C

Substance	Ω^{-1} 10^{-2}M	Ω^{-1} 10^{-3}M
$Cdpic_2Cl_2$	155	243
$Cdpic_2Br_2$	149	240
$Cdpic_2I_2$	—	202
$Cdpic_3(ClO_4)_2$.. ..	233	246

Table 2

Molecular conductances in nitromethane at 25 °C

Substance	Ω^{-1} 10^{-3}M	Substance	Ω^{-1} 3×10^{-4}M
$Cdpic_2I_2$	11	$Inpic_3I_3$ ..	188
$Cdpic_3(ClO_4)_2$ ..	143	$Inen_2I_3$ ..	61
$Inpic_2I_3$	60	$Inen_3I_3$ ..	181

ments to be made, although the iodide $Cdpic_2I_2$ at 10^{-3}M concentration in nitromethane has a conductance which is about one-sixth that of a uni-univalent electrolyte and indicates metal-halogen bonding and 6-coordinate complex formation. The colourless complex $Cdpic_3(ClO_4)_2$ was similarly prepared from aqueous solution and behaves as a bi-univalent electrolyte in both water and nitromethane in which, unlike the chloride and bromide, it is slightly soluble. A 6-coordinate structure with three molecules of chelate attached to the cadmium atom is indicated in the case of the perchlorate.

The addition of 2-picolylamine to indium halides in ethanol results in the formation of colourless trispicolylamineindium(III) salts on standing. Like the corresponding ethylenediamine complexes and unlike those with 2,2′-dipyridyl and 1,10-phenanthroline, they are hydrolysed in aqueous solution. On heating from 218 to 224 °C they decompose into the bispicolylamineindium(III) halides $Inpic_2X_3$ which are almost colourless. It was found that whilst all the indium

picolylamine complexes are almost insoluble in nitrobenzene, the iodides, like those of ethylenediamine, were sufficiently soluble in nitromethane for conductance measurements to be made. These results showed that the complexes are 6-coordinate throughout, the bis type being uni-univalent electrolytes having di-iodo metal attachment. The greater thermal and solution stability of the tris complexes of 2,2′-dipyridyl and 1,10-phenanthroline compared with those of 2-picolylamine is probably attributable to the lesser degree of π-bonding by resonance in the case of the latter chelating ligand. This would also account for the tendency for ligand substitution by more negative halide in the formation of the bis complexes of 2-picolylamine which are not formed with dipyridyl and phenanthroline. The results of the conductance measurements of both the cadmium and the indium complexes are summarized in Tables 1 and 2.

III. Experimental

The conductance measurements were made with a Philoscope Model GM4249/01 and cell GM4221. The nitromethane was purified by drying over anhydrous calcium sulphate and retaining the distillate boiling at 101–101·5 °C. Halogen was estimated with silver nitrate; indium as oxide and cadmium as sulphide gravimetrically.

(*a*) *Bis(2-picolylamine)cadmium(II) Chloride.*—$CdCl_2.2{\cdot}5H_2O$ (2·28 g; 10 mM) in water (100 ml) was treated with 2-picolylamine (3 g) with shaking, and the colourless solution allowed to stand. After several hours colourless rhombohedra crystallized out. These were filtered, washed with a little ethanol, then ether, and dried in an oven at 105 °C (yield 3·5 g, m.p. 250 °C) (Found: Cl, 17·7; Cd, 27·8%. Calc. for $C_{12}H_{16}N_4Cl_2Cd$: Cl, 17·7; Cd, 28·1%).

(*b*) *Bis(2-picolylamine)cadmium(II) Bromide.*—The above procedure was repeated with the addition of lithium bromide (3 g) in water (5 ml). The complex crystallized as colourless prisms (yield 4·4 g, m.p. 245 °C) (Found: Br, 33·0; Cd, 22·9%. Calc. for $C_{12}H_{16}N_4Br_2Cd$: Br, 32·7; Cd, 23·0%).

(*c*) *Bis(2-picolylamine)cadmium(II) Iodide.*—Procedure (*a*) was repeated adding lithium iodide (3·5 g) in water (5 ml). Colourless rhombic crystals resulted (yield 5·6 g, m.p. 217 °C) (Found: C, 25·0; H, 2·7; I, 42·7; Cd, 19·2%. Calc. for $C_{12}H_{16}N_4I_2Cd$: C, 24·7; H, 2·8; I, 43·1; Cd, 19·3%).

(*d*) *Tris(2-picolylamine)cadmium(II) Perchlorate.*—Procedure (*a*) was repeated, but sodium perchlorate (4 g) in water (8 ml) was added prior to the addition of 2-picolylamine (3·5 g). Colourless rhombohedra resulted (yield 6·1 g, m.p. 188 °C) (Found: C, 33·9; H, 3·9; Cd, 17·4%. Calc. for $C_{18}H_{24}N_6Cl_2O_8Cd$: C, 34·0; H, 3·8; Cd, 17·6%).

(*e*) *Tris(2-picolylamine)indium(III) Chloride.*—Indium(III) chloride was prepared by dissolving the metal in hot conc. HCl, evaporating to dryness, and subliming the resulting halide. Indium chloride (0·442 g; 2 mM) in ethanol (10 ml) was treated with 2-picolylamine (0·649 g; 6 mM) with stirring and the colourless solution allowed to evaporate spontaneously overnight, when small colourless crystals were formed. These were washed with a little ether and dried at 105 °C (yield 0·87 g) (Found: C, 39·2; H, 4·2; Cl, 19·4%. Calc. for $C_{18}H_{24}N_6Cl_3In$: C, 39·6; H, 4·2; Cl, 19·5%).

(*f*) *Tris(2-picolylamine)indium(III) Bromide.*—Procedure (*e*) was repeated with the addition of lithium bromide (0·5 g) in ethanol (3 ml) before adding the ligand. Fine colourless crystals resulted (yield 1·1 g) (Found: C, 31·9; H, 3·5; Br, 35·0%. Calc. for $C_{18}H_{24}N_6Br_3In$: C, 31·8; H, 3·5; Br, 35·4%).

(*g*) *Tris(2-picolylamine)indium(III) Iodide.*—Procedure (*f*) was repeated using lithium iodide (0·8 g) in lieu of lithium bromide. Colourless crystals resulted (yield 1·3 g) (Found: C, 26·7; H, 3·0; I, 46·1%. Calc. for $C_{18}H_{24}N_6I_3In$: C, 26·4; H, 3·0; I 46·5%).

(*h*) *Bis(2-picolylamine)indium(III) Chloride.*—Tris(2-picolylamine)indium(III) chloride (0·494g) was heated at 218 °C for 1 hr in an oven, when some of the base was evolved leaving a very pale

yellow residue (0·388 g). The substance was recrystallized from ethanol as almost colourless microprisms (decomp. above 300 °C) (Found: C, 33·4; H, 3·8; Cl, 24·0%. Calc. for $C_{12}H_{16}N_4Cl_3In$: C, 33·0; H, 3·7; Cl, 24·3%).

(*i*) *Bis(2-picolylamine)indium(III) Bromide.*—Procedure (*h*) was repeated at 220 °C using $Inpic_3Br_3$ (0·240 g), when almost colourless crystals resulted (0·204 g; decomp. above 300 °C) (Found: C, 25·6; H, 2·9; Br, 42·0%. Calc. for $C_{12}H_{16}N_4Br_3In$: C, 25·3; H, 2·8; Br, 42·0%).

(*j*) *Bis(2-picolylamine)indium(III) Iodide.*—Procedure (*h*) was repeated at 224 °C using $Inpic_3I_3$ (0·408 g), when almost colourless crystals resulted (0·340 g; decomp. 292 °C) (Found: C, 20·8; H, 2·3; I, 53·0%. Calc. for $C_{12}H_{16}N_4I_3In$: C, 20·5; H, 2·3; I, 53·5%).

(*k*) *Trisethylenediamineindium(III) Iodide.*—Indium(III) chloride (2 mM), together with lithium iodide (5 mM), was treated with ethylenediamine (6 mM) in ethanol and the white precipitate which formed filtered, washed with ethanol and ether, and dried at 105 °C (Found: I, 55·9; In, 17·3%. Calc. for $C_6H_{24}N_6I_3In$: I, 56·3; In, 17·0%).

(*l*) *Bisethylenediamineindium(III) Iodide.*—$Inen_3I_3$ was heated at 150 °C for 3 hr when a very pale yellow residue resulted (Found: I, 61·4; In, 18·7%. Calc. for $C_4H_{16}N_4I_3In$: I, 61·9; In, 18·6%).

IV. Acknowledgment

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STUDIES ON ESTERS OF SULPHURIC ACID

I. POSITIONS OF BOND FISSION DURING HYDROLYSIS OF DIALKYL SULPHATES IN OXYGEN-18 WATER

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Summary

The positions of bond fission during the first stage hydrolysis of dimethyl, diethyl, and di-isopropyl sulphates under acidic and alkaline conditions between 0 and 50 °C have been determined by the use of ^{18}O-enriched water. Isotopic analysis by the two independent methods, namely, mass-spectrometry and micropyknometric density determination, agreed within experimental error.

Each ester showed almost 100% alkyl–oxygen fission. $R-\overset{|}{\underset{|}{}}-O\cdot SO_2\cdot OR$, under all conditions used. The present work establishes with reasonable certainty that the results of similar work by Kursanov and Kudryavtsev (1956) on dimethyl sulphate and of Anbar *et al.* (1954) on diethyl sulphate are in error.

I. INTRODUCTION

Although the dialkyl sulphates are almost exclusively noted for their alkylating properties, that is, for reactions in which alkyl-oxygen fission occurs, $R-\overset{|}{\underset{|}{}}-O-SO_2\cdot OR$, the possibility exists that they react also by sulphur-oxygen fission, $R-O-\overset{|}{\underset{|}{}}-SO_2\cdot OR$. In the latter case sulphonation of some substance present would occur.

There are a number of references in the literature claiming that in the absence of alkali and water, dimethyl sulphate can act as a sulphonating agent. Simon and Fréjacques (1923) reported the sulphonation of anisole by dimethyl sulphate; Gibson and Vining (1923) reported the sulphonation of diphenylamine by dimethyl sulphate. Belov (1941), Belov and Shepelenkova (1941), and also Lukin (1948) have noted similar reactions. It seemed of interest therefore to determine, by use of oxygen-18 as a tracer, whether any sulphur-oxygen fission occurs when dialkyl sulphates react with water under different conditions.

While this work was in progress, two papers appeared in the literature related to the present topic. Anbar *et al.* (1954) reported that hydrolysis of diethyl sulphate in ^{18}O-water under alkaline conditions occurred with 85% carbon-oxygen fission. Kursanov and Kudryavtsev (1956) reported between 62 and 96% carbon-oxygen fission during hydrolysis of dimethyl sulphate in ^{18}O-water under acidic and alkaline conditions. Both reports appear to be in error.

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II. Experimental

(a) *Reagents*

(i) *Dimethyl Sulphate.*—Dimethyl sulphate (B.D.H.) was purified by the procedure described by Vogel and Cowan (1943) but the final distillation was carried out at 3–5 mm Hg instead of atm. pressure (n_D^{25} 1·3855). The purity of the ester was checked by hydrolysing weighed amounts to methyl sulphuric acid. The latter was titrated with carbonate-free alkali and found to correspond to 100·0% dimethyl sulphate.

(ii) *Diethyl Sulphate.*—Diethyl sulphate (B.D.H.) was purified by the same method as that used for dimethyl sulphate (n_D^{25} 1·3983). Purity was checked as above and found to be 100·0%.

(iii) *Di-isopropyl Sulphate.*—Di-isopropyl sulphate was prepared essentially by the procedure given by Levaillant (1929) and was finally purified by distillation at 1 mmHg (yield 40%; n_D^{25} 1·4057). Purity was checked as before and found to be 100·0%.

All samples of ester used in tracer studies were redistilled *in vacuo* from potassium carbonate (A.R.) immediately before use.

(iv) *Sodium Hydroxide.*—The sodium hydroxide required for hydrolysis of esters under alkaline conditions was prepared by adding purified sodium metal, contained in glass capillary tube, to the ^{18}O-water in the reaction flask. A method of filling glass tube with sodium metal is described by Kirshenbaum (1951).

(v) *Dioxan.*—Dioxan was purified by the method described by Vogel (1948) and immediately transferred to glass ampoules containing sodium wire. Air was removed and the ampoules sealed.

(b) *Reaction Mixture and Method of Hydrolysis*

In all experiments except one, approximately 10 mmoles of ester were hydrolysed in 10 ml of ^{18}O-enriched water. In the one exception (see Table 1), 10 mmoles of dimethyl sulphate were hydrolysed in 10 ml of dioxan-^{18}O-water solvent (50% w/w). The dialkyl sulphates are not very soluble in water and the hydrolysis was carried out by different methods to determine the effect of varying the reaction conditions:

(i) The ester was introduced by breaking at appropriate intervals fragile bulbs containing ester under the surface of the ^{18}O-water. In this way the system could be maintained homogeneous.

(ii) The ester was introduced in a continuous stream through a fine capillary at a rate such that homogeneous conditions were maintained. The flask containing the reaction solution was shaken throughout this process.

(iii) The ester was placed together with the ^{18}O-water in a suitably sealed reaction vessel and shaken at the desired temperature. In such cases heterogeneous conditions prevailed (except when a dioxan-water solvent was used), for the most of the reaction time. In all cases hydrolysis was carried to completion of the first stage. No hydrolysis to the second stage occurred except in the case of experiment 6, Table 2, when 0·5% second-stage hydrolysis was noted.

(c) *The Separation and Drying of Alcohols*

In experiments 1 and 2, Table 1, the methanol was separated by distillation, using a fractionating column of low hold-up. In experiment 3, involving the use of a dioxan-water solvent, the mixture was neutralized with clean magnesium metal and the volatile products were pumped through a column containing a total of 226 g of calcium sulphate (Lauder and Wilson 1961) to remove the water. The dry mixture of methanol and dioxan was then treated with sodium metal. The dioxan was removed by vacuum manipulation and a slight excess of normal water was added to the alkoxide to liberate the alcohol which was then recovered. In all other cases, the following alcohol-separation procedure was adopted: a flask containing the reaction mixture was attached to a vacuum system and most of the water and methanol were pumped off leaving

behind the alkyl sulphuric acid* or its sodium salt from the alkaline runs. The alcohol-water mixtures were then fractionated using a small still of low hold-up.

In all cases, the alcohol samples were dried with calcium sulphate using the technique described by Lauder and Wilson (1961). Yields of dry alcohols were generally 70–80% (based on dialkyl sulphate hydrolysed).

(d) Isotopic Analysis

Two methods of isotopic analysis were used : (i) The oxygen in the alcohol was converted to water (Lauder and Wilson 1959*a*) and the latter was purified (Lauder and Wilson 1959*b*). The density of the water was determined by the modified Gilfillan-Polanyi technique as described by Lauder (1959*a*). (ii) The alcohol was decomposed thermally in the presence of excess bromine (Lauder and Zerner 1959). The carbon monoxide formed was examined mass-spectrometrically (Lauder 1959*b*).

III. Results

The results are shown in Tables 1, 2, and 3. The water used in experiments 1 to 3 inclusive (Table 1) had an excess density of 356 p.p.m., while that used in all other experiments (Tables 1, 2, and 3) had an excess at-% ^{18}O equal to 0·483 and an excess density of 525 p.p.m. due to excess ^{18}O and ^{17}O (the latter contributing approximately 20 p.p.m.).

The percentage results for carbon-oxygen fission by the mass-spectrometric method of analysis are based on intercomparison measurements of the 30/28 ratios in carbon monoxide samples from alcohols and from the water used, with the mass-spectrometer operating under the same conditions. The accuracy of the mass-spectrometric measurements is about $\pm 0{\cdot}2\%$. The accuracy of the isotopic measurements by the pyknometric method is about $\pm 1{\cdot}6\%$. Isotopic measurements by the two independent methods agree within experimental error, cf. Tables 1 and 2.

IV. Discussion

The data in Tables 1, 2, and 3 show that the first stage of hydrolysis under homogeneous or heterogeneous conditions for each of the three esters investigated under acidic or alkaline conditions in the temperature range 0–50 °C occurs almost entirely by carbon-oxygen fission. The low result, 88% carbon-oxygen fission, experiment 6, Table 1, for the hydrolysis of dimethyl sulphate at 25 °C illustrates the effect of isotopic contamination resulting from the occurrence of second stage hydrolysis during pump off as explained in the footnote on this page. Again the low result (85%) for the hydrolysis of dimethyl sulphate in the presence of 3M sulphuric acid, experiment 7, Table 1, is due to the same cause.

The results reported here, ~100% carbon-oxygen fission, are similar to those reported by Ader (1949) for the positions of bond fission during hydrolysis of alkyl methane and also toluene sulphonates—as could be expected from the similarity in the chemical nature of these compounds.

Sulphur-oxygen fission during hydrolysis of sulphonate esters has been demonstrated by oxygen-18 tracer methods only when attack at carbon is

* This procedure, as subsequently shown, is not to be recommended. Hydrolysis of the alkyl sulphuric acid may occur during the pump off, even at 0 °C. Initial neutralization of the alkyl sulphuric acid obviates this source of trouble.

TABLE 1

HYDROLYSIS OF DIMETHYL SULPHATE IN ^{18}O-WATER

Expt. No.	Reaction Conditions	Temperature (°C)	Method of Hydrolysis Numbers refer to Sections II (*b*) and II (*d*)	Method of ^{18}O-Analysis Numbers refer to Sections II (*b*) and II (*d*)	Carbon-Oxygen Fission (%)
	Alkaline				
1	2N NaOH	35·0	1	1	100
2	1N NaOH	35·0	1	1	100
3	Solvolysis in dioxan-water (50% w/w)	35·0	3	1	100
4	Solvolysis	25	2	2	99·4
5*	Solvolysis	0	3	2	99·2
6*	Solvolysis	25	3	1, 2	86·3, 88·1
7*	Acidic				
	3M H_2SO_4	35	3	2	85

* Heterogeneous.

TABLE 2

HYDROLYSIS OF DIETHYL SULPHATE IN ^{18}O-WATER

Expt. No.	Reaction Conditions	Temperature (°C)	Method of Hydrolysis Numbers refer to Sections II (*b*) and II (*d*)	Method of ^{18}O-Analysis Numbers refer to Sections II (*b*) and II (*d*)	Carbon-Oxygen Fission (%)
1	Solvolysis	50	1	2	98·1
2	,, ...	35	2	2	99·1
3	,, ...	25	2	2	99·2
4*	,, ...	50	3	1, 2	98·9, 98·1
5*	,, ...	35	3	1	100
6*	,, ...	0	3	2	99·6
7*	Alkaline				
	1N NaOH	25	3	1	96·0

* Heterogeneous.

TABLE 3

HYDROLYSIS OF DI-ISOPROPYL SULPHATE IN ^{18}O-WATER

Expt. No.	Reaction Conditions	Temperature (°C)	Method of Hydrolysis Numbers refer to Sections II (*b*) and II (*d*)	Method of ^{18}O-Analysis Numbers refer to Sections II (*b*) and II (*d*)	Carbon-Oxygen Fission (%)
1	Solvolysis	10	2	2	98·5

difficult as is the case with phenyl esters. Bunton and Frei (1951) observed ~100% sulphur-oxygen fission during alkaline hydrolysis of phenyl *p*-toluene sulphonate; Bunton and Welch (1956) observed a similar result with phenyl methane sulphonate.

Kursanov and Kudryavtsev (1956) in their work on the hydrolysis of dimethyl sulphate in oxygen-18 water under heterogeneous conditions observed between 62 and 96% carbon-oxygen fission. The experimental conditions are not clearly defined. They explain their results in terms of an assumed rapid exchange of oxygen between water and the two oxygen atoms of the group, $>S\begin{matrix}\diagup\!\!O\\ \diagdown\!\!O\end{matrix}$, in in the ester molecule followed by an assumed slower reaction involving carbon-oxygen fission only. No evidence in support of these assumptions is given and moreover their data and hypothesis do not agree well requiring greater than 100% carbon-oxygen fission in three experiments out of the four carried out.

No evidence was found in the present work (in expt. 7, Table 1) for the exchange of oxygen between water and the $>S\begin{matrix}\diagup\!\!O\\ \diagdown\!\!O\end{matrix}$ group of dimethyl sulphate. It may be noted that Bunton *et al.* (1958) found no exchange of oxygen between water and the >S=O group of ethylene sulphite. Christman and Oae (1959) found no exchange of oxygen between water and diphenyl sulphone or between water and phenyl benzene sulphonate under alkaline conditions. It seems that the results of Kursanov and Kudryavtsev (1956) are in error due to the occurrence of second stage hydrolysis. In their "acidic" experiment (no temperature stated) which resulted in the observation of 62% carbon-oxygen fission the wt. % of ester was 74. This would result in the reaction mixture becoming highly acidic as hydrolysis progressed and second stage hydrolysis would be expected under these conditions (cf. footnote, p. 43).

It is of interest to note that Lewis, Mason, and Morgan (1924) found a remarkable increase in rate of hydrolysis of dimethyl sulphate under heterogeneous conditions as the wt. % of ester was increased. A change from a wt. % of ester from 0·5 to 75·0 caused the percentage hydrolysis in 3 min at 95 °C to increase from 49·9 to 96·0 for both stages. This adds further support to the interpretation given above.

It is not possible to comment on the two experiments by Kursanov and Kudryavtsev (1956) carried out under alkaline conditions because of lack of experimental detail and the ill-defined nature of the experiment.

Anbar *et al.* (1954) reported 85% carbon-oxygen fission during alkaline hydrolysis of diethyl sulphate under "reflux conditions". The experimental conditions are not completely defined and it appears that this result is in error.

Kinetic investigations to be reported in Part II of this series of papers support the view that diethyl sulphate reacts mainly by an S_N1 process. A change from solvolysis conditions, involving ~100% carbon-oxygen fission, to alkaline conditions of hydrolysis would not be expected to bring about a change in the percentage of carbon-oxygen fission observed during hydrolysis. (The solvolysis

reaction is not acid-catalysed.) It is for this reason that the result quoted by the present authors for the alkaline hydrolysis of diethyl sulphate experiment 7, Table 2, 96% carbon-oxygen fission, is considered to be too low due to experimental error.

V. Correlation of Oxygen-18 Tracer Results with Other Observations

Under heterogeneous hydrolysis conditions the possibility exists that reactions occur in the ester phase which do not occur when the ester is in aqueous solution. Some observations on reactions in the ester phase are reported in brief here.

The composition of the ester phase under typical heterogeneous conditions as used in the present work was determined. It was found that the molecular ratio of the different species present was approximately :

$$(CH_3)_2SO_4 : H_2O : CH_3OH : CH_3HSO_4 = 140 : 5 : 1 : 0{\cdot}05.$$

Experiment showed that the rate of reaction between water and dimethyl sulphate using dimethyl sulphate as the solvent is so slow that the occurrence of this reaction could not affect the oxygen-18 tracer results should this reaction occur with sulphur-oxygen fission. Again it was demonstrated using ^{14}C-methanol that the exchange,

$$(CH_3)_2SO_4 + \overset{*}{C}H_3OH \rightleftharpoons (\overset{*}{C}H_3)_2SO_4 + CH_3OH,$$

is so slow (half-life, 1–2 months) that it could not affect the oxygen-18 tracer results should it also involve sulphur-oxygen fission. Of a more subtle nature is the hydrolysis reaction undergone by methyl sulphuric acid, CH_3HSO_4. Unpublished work with Mr. B. D. Batts has shown that methyl sulphuric acid undergoes hydrolysis very much more rapidly in solvents of low polarity containing a trace of water than in aqueous solution. The reaction is reversible and sulphur-oxygen fission is probably involved (cf. Burstein and Lieberman 1958). It was thought possible that, in a heterogeneous system, alcohol produced by carbon-oxygen fission in the aqueous phase could move into the ester phase and exchange there with methyl sulphuric acid via a process involving sulphur-oxygen fission,

$$CH_3{\cdot}O{\cdot}SO_2OH + CH_3\overset{*}{O}H \rightleftharpoons CH_3{\cdot}\overset{*}{O}{\cdot}SO_2OH + CH_3OH.$$

That this reaction does not lead to any isotopic dilution of the alcohol produced in the oxygen-18 tracer experiments under heterogeneous conditions was demonstrated by adding some ^{14}C-methanol to a heterogeneous reaction mixture undergoing hydrolysis. No activity above background was found in the methyl sulphuric acid at the end of the hydrolysis. It may be concluded therefore that any major deviation of the oxygen-18 tracer results from ~100% carbon-oxygen fission for the hydrolysis of dimethyl sulphate cannot be attributed to the nature of the system—homogeneous or heterogeneous.

The reports on sulphonation by dimethyl sulphate mentioned in Section I are of interest in connection with the present work. These reactions have been

observed in the absence of water and alkali. It is under these conditions that methyl sulphuric acid is most reactive. (Reactions of the alkyl sulphuric acids will be discussed in a subsequent paper.) It seems reasonable to attribute the formation of sulphonated products in many cases to a secondary reaction—to a reaction of methyl sulphuric acid produced during an initial methylation process, for example,

$$(CH_3)_2SO_4+(C_6H_5)_2NH \rightarrow (C_6H_5)_2N\cdot CH_3+CH_3HSO_4$$

rather than to a primary reaction of dimethyl sulphate itself. In other cases, particularly in the work of Belov (1941) and of Belov and Shepelenkova (1941) carried out at higher temperatures, dimethyl sulphate may decompose to give products with sulphonating properties.

VI. Acknowledgments

It is a pleasure to acknowledge a generous gift of oxygen-18-enriched water from Professor D. R. Llewellyn, Auckland University, New Zealand. Dr. M. D. Sutherland advised us on distillation procedures. Mr. B. D. Batts advised us on the properties of the alkyl sulphuric acids. We thank Mr. D. Kerr for experimental assistance. One of us (B.Z.) acknowledges the award of a scholarship by the Australian Atomic Energy Commission.

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METHYLSTEROIDS*

VII. FACTORS AFFECTING THE HYDROLYSIS OF 7α- AND 11β-ACETOXYLANOSTANES

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Summary

Doubly bonded methylene groups have been introduced into 7α- and 11β-acetoxylanostanes and the rate of hydrolysis compared with compounds having a hydroxy or carbonyl group at the same position as the methylene group. It was found that methylene groups facilitate hydrolysis of the hindered acetoxy groups in the same way, but not to the same extent, as carbonyl groups. It is concluded that the facilitation in each case results from a conformational disturbance, but that there is some other factor involved in carbonyl facilitation. It was not possible to demonstrate a similar effect resulting from steric crowding of substituents.

I. INTRODUCTION

Previous work (Barnes 1958) has shown that the rate of hydrolysis of acetoxy groups at hindered axial positions on the lanostane skeleton is enhanced by the presence of carbonyl groups in rings other than that containing the acetoxy group. Thus although the 11β-acetoxy group is usually completely resistant to basic hydrolysis it reacts under mild conditions when a 7-oxo-group is present in the same molecule, and even more readily in the presence of a 3,7-dioxo-grouping. Similarly, a 7α-acetoxy group which normally hydrolyses slowly, reacts readily in the presence of either a 3- or 11-oxo-group. It was concluded that these very marked effects were the result of partial loss of axial character of the acetoxy groups or their hindering methyl groups, caused by changes in molecular conformation produced by the carbonyl groups and transmitted along the ring system.

So that this explanation could be checked, it seemed desirable to produce similar conformational distortions by means of analogous effects in which the possibility of polar effects arising is precluded. We have therefore prepared analogues of some activated compounds in which the oxygen atom of the activating carbonyl groups is replaced by an exocyclic methylene group.

II. ACETATE HYDROLYSIS

Because the effect of the 7-carbonyl group on the hydrolysis of the 11β-acetoxy group is so marked, attempts were first made to introduce an exocyclic methylene group at the 7-position. As expected the 7-oxo-group was too inert to react with methylene triphenylphosphine, and although a pair of 7-epimers was obtained from the reaction of methylmagnesium iodide with 3β-acetoxy-

* For Part VI of this series see Barnes (1959).

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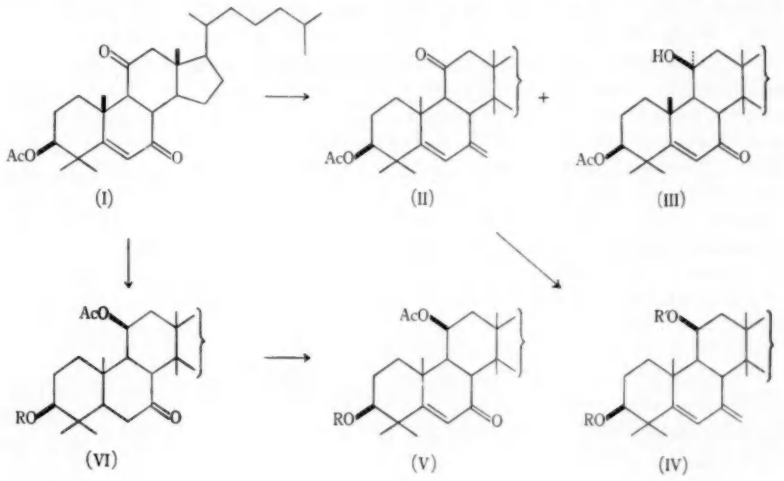
lanostan-7,11-dione, it was not found possible to dehydrate either to a doubly bonded methylene. Therefore no simple 7-methylene compound has yet been obtained, but a 7-methylene compound having a conjugated double bond was prepared in the following manner.

Methylmagnesium iodide reacted with 3β-acetoxylanost-5-en-7,11-dione (I)* to give after acetylation and chromatography two compounds. The more easily eluted is formulated as 3β-acetoxy-7-methylenelanost-5-en-11-one (II) by analogy with the corresponding reaction in the steroid series (Bann *et al.* 1936), and because of its ultraviolet absorption maximum (244 mμ) and the presence in



the infrared absorption spectrum of absorption due to doubly bonded methylene (885 cm^{-1}), acetate carbonyl (1740 cm^{-1}), and six-membered ring carbonyl (1710 cm^{-1}). The more strongly absorbed fractions gave 3β-acetoxy-11β-hydroxy-11-methyl-lanost-5-en-7-one (III).† That reaction had occurred with the carbonyl group at the 11-position rather than the conjugated carbonyl group was shown by loss of six-membered ring carbonyl absorption (1705 cm^{-1}) and presence of bands at 240 mμ and 1667 cm^{-1} attributed to the original enone system. The configuration at the 11-position is assigned in accordance with the Cram and Elhafez (1952) rule of asymmetric induction.

* This compound has twice been stated to resist hydrolysis by acid or base (Doree, McGhie, and Kurzer 1949; Cavalla, McGhie, and Pradhan 1951). We considered this to be unlikely though it seemed possible that some unusual property may be associated with the vinylogous β-hydroxyketone system. In fact a gelatinous hydroxy compound was formed normally, and was characterized by benzoylation and by oxidation to the 3-oxo-compound (see Section V).

† It is possible that other hydroxy-containing products were present, since the crude (III) showed a small band at 1705 cm^{-1}. This could have arisen from a 7-hydroxy-7-methyl-11-oxo-compound, but if so the quantity was small and no product other than (III) was isolated.

Reduction of the carbonyl group in the diene (II) with lithium aluminium hydride followed by acetylation with acetic anhydride in pyridine gave 3β-acetoxy-7-methylenelanost-5-en-11β-ol (IV ; R=Ac, R′=H) the expected β-configuration of the hydroxy group being confirmed by its resistance to acetylation. When acetylated with acetic anhydride and toluene-*p*-sulphonic acid the diacetate (IV ; R=R′=Ac) was obtained as shown by the absence of hydroxyl and carbonyl absorption other than acetate carbonyl in the infrared. That the diene system remained unchanged was shown by the ultraviolet absorption spectrum (maximum at 246 mμ) and the presence of a band (890 cm^{-1}) in the infrared attributed to the exocyclic methylene group. Hydrolysis of the diacetate (IV ; R=R′=Ac) for a short time gave the 3β-hydroxy compound (IV ; R=H, R′=Ac).

To determine the effect of the conjugated diene system in (IV) on the rate of hydrolysis of the 11β-acetoxy group, it was necessary to have the conjugated enone system as in (V) for comparison. However, reduction of (I) with lithium aluminium hydride followed by complete acetylation gave as sole product 3β,11β-diacetoxylanostan-7-one (VI ; R=Ac) identified by comparison of infrared spectra and mixed melting point with material produced by other methods (Barnes 1958). This result is in contrast to the lithium aluminium hydride reduction of steroidal 5-en-7-ones which gives the allylic alcohol by reduction of the carbonyl group (Fieser, Fieser, and Chakravarti 1949). It is no doubt due to the additional hindrance to the transition state produced by the 14α-methyl group, as similar reductions of ethylenic bonds in other alicyclic enones are known, but are rare (Mousseron *et al.* 1952 ; Halsall and Moyle 1960).

As with other 7-oxolanosterol derivatives (McGhie, Pradhan, and Ross 1953) the double bond of the required 5-en-7-one system was easily introduced by selenium dioxide oxidation of (VI ; R=Ac) to give (V ; R=Ac) which on partial hydrolysis gave (V ; R=H).

Treatment of (IV ; R=H, R′=Ac) and (V ; R=H) with ethanolic alkali under identical conditions showed (Table 1) that reaction occurred in each case, but that it was slower with the methylene compound (IV) than with the ketone (V). That the chromophore was unchanged by the hydrolytic treatment was shown by the spectroscopic properties of the isolated amorphous hydroxy compound.

Although this result shows that conformational changes may occur with the 5-en-7-one and the corresponding conjugated diene, the compounds are not strictly comparable with the saturated oxoacetates previously studied. To obtain an exocyclic methylene group free from the complication of a conjugated double bond, recourse was had to the Wittig reaction (Sondheimer and Mechoulan 1958) at the 3-position.

Reaction of 11β-acetoxylanostan-3,7-dione (VII) (Barnes 1958) with methylene triphenylphosphine gave, after chromatography, 11β-acetoxy-3-methylenelanostan-7-one (VIII) (m.p. 152 °C) which had an infrared absorption spectrum (CS_2) differing from that of the starting material by the presence of an intense band at 895 cm^{-1} due to the methylene group, and by the ketone carbonyl band (1705 cm^{-1}) being less intense than the acetate carbonyl (1730 cm^{-1}).

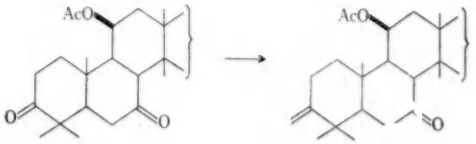
TABLE 1

HYDROLYSIS OF ACETOXYLANOSTANES IN 0·3N ETHANOLIC POTASSIUM HYDROXIDE

Compound	No. of Acetoxyls Hydrolysed in :	
	1 Hour	2 Hours
11β-Acetoxy-7-methylenelanost-5-en-3β-ol (IV ; R=H, R′=Ac)	0·2	
11β-Acetoxy-3β-hydroxylanost-5-en-7-one (V ; R=H)	0·3	
11β-Acetoxy-3-methylenelanostan-7-one (VIII)		0·4
11β-Acetoxylanostan-3,7-dione (VII)		0·8
11β-Acetoxy-3β-hydroxylanostan-7-one (VI ; R=H)		0·3
7α,11β-Diacetoxy-3-methylenelanostane (XI)	0·7*	
7α,11β-Diacetoxylanostan-3-one (X)	0·9*	
7α,11β-Diacetoxylanostan-3β-ol (IX ; R=H, R′=Ac)	0·2*	
7α-Acetoxylanostan-3-one	0·8*	
7α-Acetoxylanostan-3β-ol (XIII)	0·4	

* With added benzene.

When the reaction product was purified only by crystallization it had a constant melting point (150 °C) not very different from and not depressed when mixed with the pure methylene compound. The infrared spectrum was similar to that of pure (VIII), but the two carbonyl bands were of equal intensity. Elementary analysis indicated that it was probably an equimolecular mixture of dione and methylene compound, and these two compounds were obtained in chromatography.

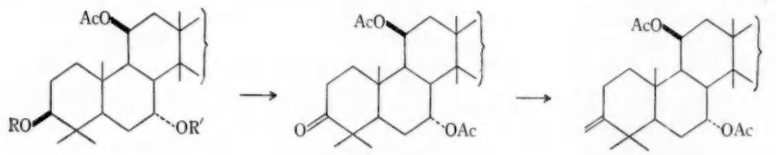


(VII) (VIII)

Treatment of (VII) and (VIII) under identical conditions with ethanolic alkali showed (Table 1) that the latter, having a 3-methylene group, was hydrolysed less readily than (VII), which has a 3-oxo-group, but at a slightly greater rate than the 3-hydroxy compound (VI ; R=H). That the methylene group was stable to the conditions used was shown by recovery of starting material (VIII) on acetylation of the hydrolysis product. Hence the methylene group does produce a facilitation of hydrolysis, but again not to the same extent as a carbonyl group. It was felt that this result needed further confirmation because the effect being observed was due to a small increment from the methylene

group being superimposed on the existing carbonyl facilitation resulting from the 7-oxo-group. So that the effect of the methylene group could be dissociated from that of the carbonyl group an examination was made of facilitation of hydrolysis of 7α-acetates.

The triacetate, 3β,7α,11β-triacetoxylanostane (IX ; R=R′=Ac) previously prepared (Barnes and Palmer 1957) by lithium aluminium hydride reduction of 3β,7α-diacetoxylanostan-11-one followed by acetylation, was also prepared by acetylation of 3β,11β-diacetoxylanostan-7α-ol (IX ; R=Ac, R′=H) which resulted from catalytic reduction of (VI ; R=Ac). Brief treatment of (IX ;



(IX) (X) (XI)

R=R′=Ac) with ethanolic alkali gave the alcohol (IX ; R=H, R′=Ac) which oxidized to the 3-ketone (X) (Barnes and Palmer 1957). This reacted with methylene triphenylphosphine to give 7α,11β-diacetoxy-3-methylenelanostane (XI) the structure of which was confirmed by absorption at 890 cm^{-1} due to the doubly bonded methylene.

Treatment of (IX ; R=H, R′=Ac), (X), and (XI) under identical conditions with ethanolic alkali showed (Table 1) that the latter, having a 3-methylene group, hydrolysed more rapidly than (IX ; R=H, R′=Ac), having a 3-hydroxy group, but slower than the 3-oxo-compound (X).

III. Conclusions

The fact that the methylene compounds (VIII) and (XI) were hydrolysed more readily than the hydroxy compounds (VI) and (IX) is taken to confirm the proposal for a conformational transmission effect, since it is unlikely that polar effects would be associated with the ethylenic bond. But since the carbonyl compounds (V), (VII), and (X) are hydrolysed more readily than the methylene analogues (IV), (VIII), and (XI) there is evidently some other factor associated with the carbonyl group.

Somewhat similar results have been obtained by Barton *et al.* (1960), by using their method of condensation of 3-ketones with benzaldehyde. Recently Fox, Origoni, and Smith (1960) have observed that acetylation of some 11β-hydroxy steroids (e.g. hydrocortisone 21-acetate) is possible under relatively mild conditions. This is probably also a conformational effect due largely to the double bond of the 4-en-3-one system. It would be interesting to compare that reaction result with that of the similar saturated ketone, where no acetylation would be expected.

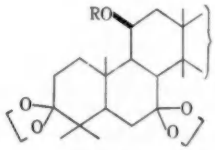
Finally, the formation of (III) by Grignard reaction with (I) is probably another example of the ability of conformational changes to affect the reactivity

of functional groups. Normally the 11-oxo-group is inert to the Grignard reagent, but in the presence of the 5-en-7-one system the 11-oxo-group reacted in preference to the conjugated carbonyl group.

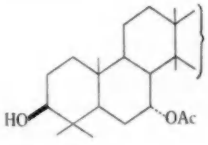
IV. Steric Compression Effects

It was first thought that the steric crowding which causes the inertness of substituents at the 7α- and 11β-positions could also cause distortions of the ring system similar to those produced by carbonyl groups. This view arose partly from unpublished qualitative observations on differences in the manner of dehydration of the epimeric 11-hydroxyl groups depending on whether or not the 7-position was disubstituted. It would be expected that interactions between 7-substituents, particularly at the 7α-position, and the methyl group on C_{14}, should be sufficient to influence reactivity at the 11-position.

A convenient test for this hypothesis seemed possible by hydrolysis of the acetate of 3,3,7,7-diethylenedioxylanostan-11β-ol (XII; R=H) (Barnes 1958). This acetate had not been prepared because application of the acetic anhydride–toluenesulphonic acid method caused simultaneous acetylation and deketalization.



(XII)



(XIII)

The acetate (XII; R=Ac) was found to form, however, when acetyl chloride was used in dimethylaniline solution. Treatment of this acetate with refluxing methanolic potassium hydroxide (5%) for 1 hr caused no obvious hydrolysis, as shown by recovery of pure starting material. Hence distortion was not demonstrated. However, if this effect does operate, it would be more likely to arise from the severe interactions between an 11β-substituent and the two angular methyl groups, and be detected by a change in the rate of hydrolysis of the 7α-acetoxy group. A comparison was therefore made of the degree of hydrolysis under similar conditions of (IX; R=H, R′=Ac), which has an 11β-acetoxy group, and (XIII) (Barnes and Palmer 1957), which has an unsubstituted 11-position, with the expectation that the former would hydrolyse more readily. In fact this was not the case and distortions arising from steric repulsions have not been demonstrated.

V. Experimental

(*a*) *General.*—Melting points are corrected and taken in open capillaries unless stated otherwise. Elementary analyses were done at the C.S.I.R.O. and University of Melbourne Microanalytical Laboratory. Rotations were measured at a concentration of 1–2 g in 100 ml in chloroform solution at 20±2 °C. Ultraviolet absorption spectra were measured in ethanol solution, and infrared spectra as indicated. Light petroleum refers to the fraction b.p. 60–80 °C.

When no method of working up a reaction product is indicated, it is understood that the usual method of ether extraction, washing of the ether solution to neutrality and evaporation to dryness at atmospheric pressure was followed.

(*b*) *Characterization of 3β-Acetoxylanost-5-en-7,11-dione* (*I*).—(i) The acetate (I ; 300 mg) was hydrolysed by refluxing in methanolic KOH (5% ; 50 ml) for 1 hr. The product formed gels from light petroleum and was very soluble in methanol ; it was evidently 3-hydroxylanost-5-en-7,11-dione, and had $\lambda_{max.}$ 238 mμ ε 11,000 (Found : C, 79·1 ; H, 10·7%. Calc. for $C_{30}H_{48}O_3$: C, 78·9 ; H, 10·6%). The hydroxy compound (60 mg) was benzoylated by heating on the steam-bath in pyridine (2 ml) and benzoyl chloride (0·5 ml) for 30 min. Crystallization of the product from chloroform–methanol gave *3β-benzoyloxylanost-5-en-7,11-dione*, m.p. 187–189 °C, $[\alpha]_D$ +45°, $\lambda_{max.}$ 234 mμ ε 24,000 (Found : C, 79·1 ; H, 9·5%. Calc. for $C_{37}H_{52}O_4$: C, 79·0 ; H, 9·4%).

(ii) The acetate (I; 300 mg) was hydrolysed by refluxing in ethanolic HCl (25 ml ethanol, 1 ml conc. HCl) for 3 hr. The product after benzoylation as in part (*b*) (i) had the same absorption in the u.v. and the same m.p. undepressed on mixing with the benzoate obtained previously.

(iii) The hydroxy compound (300 mg) obtained as in (*b*) (i), except that the time of reflux was reduced to 10 min, was dissolved in chloroform (10 ml) and acetic acid (50 ml) added followed by a solution of chromium trioxide (500 mg) in water (1 ml).

After standing 2 hr at room temperature the product was separated and crystallized from ethanol to give *lanost-5-en-3,7,11-trione*, m.p. 164 °C, $[\alpha]_D$ +55°, $\lambda_{max.}$ 236 mμ ε 10,000, maxima (CCl_4) at 1710, 1675, 1620 (small) cm^{-1} (Found : C, 79·5 ; H, 10·1%. Calc. for $C_{30}H_{46}O_3$: C, 79·2 ; H, 10·2%).

(*c*) *Reaction of 3β-Acetoxylanost-5-en-7,11-dione* (*I*) *with Methylmagnesium Iodide*.—The dione (I ; 1·3 g) in anhydrous ether (200 ml) was added to methylmagnesium iodide (from magnesium 1·3 g) and methyl iodide (4 ml) in ether (100 ml) and refluxed for 3 hr. The product was isolated after pouring onto ice and ammonium chloride and acetylated with acetic anhydride and pyridine on the steam-bath for 30 min. Chromatography over alumina gave in the light petroleum eluates (370 mg) *3β-acetoxy-7-methylenelanost-5-en-11-one* (*II*), which crystallized from methanol to have m.p. 87–89 °C, $[\alpha]_D$ +89°, $\lambda_{max.}$ 244 mμ ε 19,000, maxima (CS_2) 885, 1245, 1710, 1740 cm^{-1}. (Found : C, 80·0 ; H, 10·2%. Calc. for $C_{33}H_{52}O_3$: C, 79·8 ; H, 10·6%). Benzene eluted *3β-acetoxy-11β-hydroxy-11-methyl-lanost-5-en-7-one* (*III*) which crystallized from methanol to have m.p. 229 °C, $[\alpha]_D$ −24°, maxima (CS_2, 5 mg in 0·75 ml) at 1243, 1667, 1740, and 3615 cm^{-1} (Found : C, 77·1 ; H, 10·6%. Calc. for $C_{33}H_{54}O_4$: C, 77·0 ; H, 10·6%).

(*d*) *3β-Acetoxy-7-methylenelanost-5-en-11β-ol* (*IV ; R=Ac, R′=H*) *and Derivatives*.—The dienone (II ; 400 mg) in anhydrous ether (400 ml) was reduced by the addition of lithium aluminium hydride (500 mg) and refluxing for 2 hr. Portion (20 mg) of the product was acetylated with pyridine and acetic anhydride on the steam-bath for 15 min giving *3β-acetoxy-7-methylenelanost-5-en-11β-ol* (*IV ; R=Ac, R′=H*) which crystallized from methanol to have m.p. 218–221 °C, $[\alpha]_D$ +126°, maxima (Nujol) at 3560, 1720, 1270, and 885 cm^{-1} (Found : C, 79·25 ; H, 10·7%. Calc. for $C_{33}H_{54}O_3$: C, 79·5 ; H, 10·9%). The remainder of the reduction product was acetylated with acetic anhydride–acetic acid–toluene-*p*-sulphonic acid overnight at room temperature, and gave *3β,11β-diacetoxy-7-methylenelanost-5-ene* (*IV ; R=R′=Ac*) crystallizing from methanol to have m.p. 204–209 °C (open tube), 208–210 °C (sealed evacuated tube), $[\alpha]_D$ +125°, $\lambda_{max.}$ 246 mμ ε 14,500, maxima (Nujol) 890, 1245, 1725 cm^{-1} (Found : C, 78·0 ; H, 10·3%. Calc. for $C_{35}H_{54}O_4$: C, 78·0 ; H, 10·1%). The diacetate (IV ; R=R′=Ac) was part hydrolysed by 10 min treatment with refluxing 0·3N ethanolic KOH to give *11β-acetoxy-7-methylenelanost-5-ene-3β-ol* (*IV ; R=H, R′=Ac*) crystallizing from chloroform–methanol to have m.p. 182–184 °C, maxima (Nujol) at 3540, 1725, 1265, 885 cm^{-1} (Found : C, 79·7 ; H, 10·9%. Calc. for $C_{33}H_{54}O_3$: C, 79·5 ; H, 10·9%).

(*e*) *3β,11β-Diacetoxylanostan-7-one* (*VI ; R=Ac*).—3β-Acetoxylanost-5-en-7,11-dione (I; 250 mg) in anhydrous ether (200 ml) was reduced by the addition of lithium aluminium hydride (300 mg) followed by refluxing for 2 hr. Excess reagent was decomposed with ethyl acetate, and the isolated product acetylated by standing overnight at room temperature in a solution of acetic acid, acetic anhydride, and toluene-*p*-sulphonic acid. Filtration through alumina gave

3β,11β-diacetoxylanostan-7-one (VI; R=Ac) (200 mg), m.p. 217–218 °C, $[\alpha]_D$ +56°, correct analysis, and mixed m.p. and i.r. spectrum identical with that produced by a different method (Barnes 1958).

(*f*) *3β,11β-Diacetoxylanost-5-en-7-one* (*V; R=Ac*).—3β,11β-Diacetoxylanostan-7-one (VI; R=Ac) (1 g) in acetic acid (75 ml) was oxidized under reflux for 20 min with selenium dioxide (2 g). After removal of elementary selenium and filtration through alumina the product crystallized from light petroleum or methanol to give *3β,11β-diacetoxylanost-5-en-7-one* (*V; R=Ac*), m.p. 201–203 °C, $[\alpha]_D$ +6°, $\lambda_{max.}$ 233 mμ ε 18,000, maxima (CS_2) at 1245, 1665, 1735 cm^{-1}. Treatment of the diacetate (V; R=Ac) (350 mg) with refluxing 0·3N ethanolic KOH solution (50 ml) for 10 min gave *11β-acetoxy-3β-hydroxylanost-5-en-7-one* (*V; R=H*) crystallizing from methanol to have m.p. 190–191 °C (Found: C, 76·9; H, 10·5%. Calc. for $C_{32}H_{52}O_4$: C, 76·7; H, 10·5%).

(*g*) *11β-Acetoxy-3-methylenelanostan-7-one* (*VIII*).—A solution of n-butyl-lithium (12 ml; 1·2N) was added to a suspension of methyl triphenylphosphonium bromide (4·8 g) in anhydrous ether (70 ml) with vigorous stirring under nitrogen. Stirring was continued for a further 2 hr when 11β-acetoxylanostane-3,7-dione (VII; 1 g) in anhydrous tetrahydrofuran (50 ml) was added over a period of 15 min. After standing overnight at room temperature the solution was refluxed for 6 hr, then worked up. Crystallization of the product from chloroform–methanol or light petroleum gave well-defined crystals having m.p. 150–151 °C, $[\alpha]_D$ +22°, maxima (CS_2) 895, 1705, 1730 cm^{-1}, the i.r. absorption spectrum differing significantly from that of the starting material only by the presence of the intense band at 895 cm^{-1} (Found (after crystallization from chloroform–methanol): C, 78·2; H, 10·4%; (after crystallization from light petroleum): C, 78·4; H, 10·4%. Calc. for a 1:1 mixture of 11β-acetoxy-3-methylenelanostan-7-one: 11β-acetoxylanostane-3,7-dione: C, 78·1; H, 10·7%).

The product from a duplicate reaction was chromatographed over alumina to give in the light petroleum eluates 11β-acetoxy-3-methylenelanostan-7-one (VIII), crystallizing from chloroform–methanol, to have m.p. 152·5–153·5 °C, $[\alpha]_D$ +22°, maxima (CS_2) 895, 1705, 1730 cm^{-1} (Found: C, 79·6; H, 10·8%. Calc. for $C_{33}H_{54}O_3$: C, 79·5; H, 10·9%).

(*h*) *3β,11β-Diacetoxylanostan-7α-ol* (*IX; R=Ac, R′=H*).—3β-Acetoxylanostane-7,11-dione (24 g) was dissolved in glacial acetic acid (1000 ml) and agitated in an atmosphere of hydrogen with several grams of platinum for 3 days. The 7α-hydroxy compound was filtered directly from the solution and a further quantity could be obtained by chromatography of the material remaining in solution. The compound obtained in this way had m.p. 256–261 °C and was undepressed on mixed m.p. and had an identical i.r. spectrum with (IX; R=Ac, R′=H) obtained by another method (Barnes and Palmer 1957).

(*i*) *7α,11β-Diacetoxy-3-methylenelanostane* (*XI*).—A solution of n-butyl-lithium (12 ml; 1·4N) was added to a suspension of methyl triphenylphosphonium bromide (4·8 g) in anhydrous ether (70 ml) with vigorous stirring under nitrogen. Stirring was continued for a further 2 hr, when 7α,11β-diacetoxylanostan-3-one (X; 1 g) in anhydrous tetrahydrofuran was added over a period of 15 min with vigorous stirring. Heating under reflux was continued for 8 hr, and the product acetylated with acetic anhydride in pyridine on the steam-bath. Chromatography over alumina gave in the petrol–benzene eluates (200 mg), *7α,11β-diacetoxy-3-methylenelanostane* (*XI*), crystallizing from methanol to have m.p. 223–226 °C, $[\alpha]_D$ −25°, maxima (CS_2) 1735, 1640, 1245, 892 cm^{-1} (Found: C, 77·1; H, 10·4%. Calc. for $C_{35}H_{56}O_4$: C, 77·4; H, 10·8%).

(*j*) *3,3,7,7-Diethylenedioxylanostan-11β-yl Acetate* (*XII; R=Ac*).—The 11β-hydroxy compound (XII; R=H; Barnes 1958) (1·5 g), dimethylaniline (40 ml), and acetyl chloride (6 ml) were heated together on the steam-bath for 1·5 hr. The product was filtered through alumina in benzene solution and crystallized from chloroform–methanol to give *3,3,7,7-diethylenedioxylanostan-11β-yl acetate* (*XII; R=Ac*), m.p. 216–218 °C, $[\alpha]_D$ +12°, maxima (Nujol) 1250, 1720 cm^{-1} (Found: C, 73·1; H, 10·2%. Calc. for $C_{36}H_{60}O_6$: C, 73·4; H, 10·3%). The acetoxy compound (50 mg) was dissolved in acetic acid and the solution stood on the steam-bath for 10 min. The product after crystallization had m.p. undepressed by, and i.r. spectrum identical with that of, 11β-acetoxylanostane-3,7-dione (VII; Barnes 1958).

(*k*) *Quantitative Hydrolyses.*—These were carried out as described previously (Barnes 1958). The hydrolyses marked by an asterisk in Table 1 involved the addition of 1 ml of benzene before the alkali, in order to dissolve the compound.

Note added in proof January 11, 1961.—Recently Schwarz, Hermaner, and Trojuner (*Chem. & Ind.* **1960**: 1212) have obtained evidence which shows the operation of inductive effects over long distances in the steroids.

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UNIMOLECULAR FILMS FROM CERTAIN ANTHRACENE DERIVATIVES

By F. H. C. STEWART*

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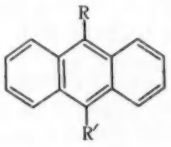
Summary

Monolayers of varying stability are formed by a series of alcohols, carboxylic acids, and other derivatives containing an anthracene nucleus. Surface pressure-area curves have been obtained for the more stable films. The limiting areas and other properties of the films are discussed with reference to the molecular configurations of the compounds.

I. INTRODUCTION

The literature on unimolecular films from compounds containing aromatic ring systems is not very extensive. Adam (1923) and Adam, Berry, and Turner (1928) studied the long-chain *p*-alkylphenols and the corresponding anilines, anisoles, and resorcinols. This work established that the benzene ring occupied an area of some 23 Å^2 in a compressed monolayer. Later Ställberg-Stenhagen and Stenhagen (1941) examined two long-chain ω-phenyl substituted carboxylic acids with similar results. Giles and Neustadter (1952) found that stable



(I)

Fig. 1.—Anthracene derivatives examined for surface activity.

(*a*) R=H; R′=CH_2OH.
(*b*) R=H; R′=$(CH_2)_3OH$.
(*c*) R=H; R′=$(CH_2)_5OH$.
(*d*) R=C_4H_9; R′=CH_2OH.
(*e*) R=H; R′=$(CH_2)_5O{\cdot}CO{\cdot}CH_3$.
(*f*) R=C_4H_9; R′=$(CH_2)_2CO_2H$.
(*g*) R=C_4H_9; R′=$(CH_2)_3OH$.
(*h*) R=$C_{12}H_{25}$; R′=CHO.
(*i*) R=$C_{12}H_{25}$; R′=CH_2OH.
(*j*) R=$C_{12}H_{25}$; R′=$(CH_2)_2CO_2H$.
(*k*) R=$C_{12}H_{25}$; R′=$(CH_2)_3OH$.

condensed monolayers were formed by various long-chain alkyl substituted azo and hydroxyazo compounds, and a range of these materials incorporating the benzene, naphthalene, anthracene, and phenanthrene ring systems, respectively, was investigated. These films were found to have limiting areas in the range 30–55 Å^2. More recently Hibberd and Alexander (1959) examined some aromatic alcohols with sterically hindered hydroxyl groups.

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In the present work the surface activity of the group of *meso*-substituted anthracene derivatives represented in Figure 1 has been investigated. The preparation of these compounds has already been described (Stewart 1960). It was hoped to determine the general effect of the presence of an anthracene nucleus on monolayer behaviour in connection with the study of some surface reactions involving this reactive aromatic system.

II. Experimental Methods

The surface pressure-area curves were obtained at room temperature (20 ± 1 °C) using an ordinary surface trough enclosed by a Polythene-covered framework. The surface pressure was measured by the Wilhelmy plate method.

The compounds were best spread from benzene solution (1 mg/ml) using an Agla micrometer syringe. The more soluble compounds could also be spread satisfactorily from light petroleum solution. The carboxylic acids were spread on 0·01N hydrochloric acid, and the remainder of the compounds on distilled water.

III. Results

Most of the compounds studied formed monolayers, which, however, varied greatly in stability and collapse behaviour. A summary of the results obtained is collected in Table 1, where the compounds are arranged in order of increasing number of saturated carbon atoms. The corresponding melting points are also given for comparison. Surface pressure-area curves for the more stable monolayers of Table 1 are reproduced in Figure 2. The form of these curves, as well as the agreement of the observed limiting areas with values deduced from molecular models (Section IV), suggests that the films are of the condensed type.

Some of the less stable films gave quite reproducible limiting areas at which the surface pressure increased markedly, although falling back to zero more or less rapidly. Thus, the monolayer of 9-dodecyl-10-hydroxymethylanthracene (Fig. 2*C*) could be compressed to over 20 dynes cm^{-1} before collapse became very rapid.

The film of 9-dodecyl-10-anthraldehyde (Fig. 2*D*) collapsed very suddenly at about 1 dynes cm^{-1}, but this behaviour was not observed with any of the other monolayers which either failed to exhibit a definite collapse pressure as mentioned above or else attained a constant collapse pressure which did not vary with further compression. This latter behaviour is exemplified by 9-ε-acetoxyamylanthracene (Fig. 2*E*). On decompression from the onset of collapse these films exhibited good reversibility along the original pressure-area curve.

The compounds with less than five saturated carbon atoms appeared to form transitory films which dissolved readily in the aqueous substrate.

IV. Discussion

The results recorded in Table 1 indicate that the formation of a stable monolayer in this series of anthracene derivatives requires the presence of at least thirteen saturated carbon atoms in the molecule. Apparently the C_{14} anthracene nucleus contributes very little to the hydrophobic character of the

hydrocarbon portion of the molecule. On the other hand, the failure of the compounds towards the top of Table 1 to form stable monolayers, owing to the solubility of the films in the aqueous phase, suggests that the anthracene nucleus has appreciable hydrophilic character. There does not seem to be any marked correlation between the melting points of the compounds and monolayer stability (cf. Table 1).

TABLE 1

MONOLAYERS FROM SOME ANTHRACENE DERIVATIVES

Compound	Number of Saturated Carbon Atoms	Melting Point (°C)	Limiting Area (Å²)	Comments
9-Hydroxymethylanthracene (I (*a*)) ..	1	156	—	No film formed
9-γ-Hydroxypropylanthracene (I (*b*))	3	97·5	—	Very fugitive film
9-ε-Hydroxyamylanthracene (I (*c*)) ..	5	93·5	~28	Very unstable
9-n-Butyl-10-hydroxymethylanthracene (I (*d*))	5	194	—	Very unstable
9-ε-Acetoxyamylanthracene (I (*e*)) ..	6	71	36	Stable low-pressure film
β-(9-n-Butyl-10-anthryl)propionic acid (I (*f*))	6	153	~37	Unstable
9-n-Butyl-10-γ-hydroxypropyl-anthracene (I (*g*))	7	114·5	42	Unstable
9-Dodecyl-10-anthraldehyde (I (*h*)) ..	12	80	43	Fairly stable at low pressures
9-Dodecyl-10-hydroxymethylanthracene (I (*i*))	13	144	46	Fairly stable
β-(9-Dodecyl-10-anthryl)propionic acid (I (*j*))	14	112	48	Stable
9-Dodecyl-10-γ-hydroxypropyl-anthracene (I (*k*))	15	94	47	Stable

The more stable monolayers have limiting areas in the range 45–48 Å², and are quite compressible (Fig. 2), but 9-ε-acetoxyamylanthracene (Fig. 2*E*) is exceptional in that the limiting area is considerably smaller (~36 Å²). The area occupied by the molecules should be governed to a large extent by the steric requirements of the anthracene nucleus which is the most bulky single grouping present. The dimensions of the anthracene molecule in the crystal are 11·2 Å long, 6·7 Å wide, and 3·4 Å thick (Robertson 1953). These values lead to three basic cross sectional areas for the nucleus, namely, 23 Å² along the short axis of

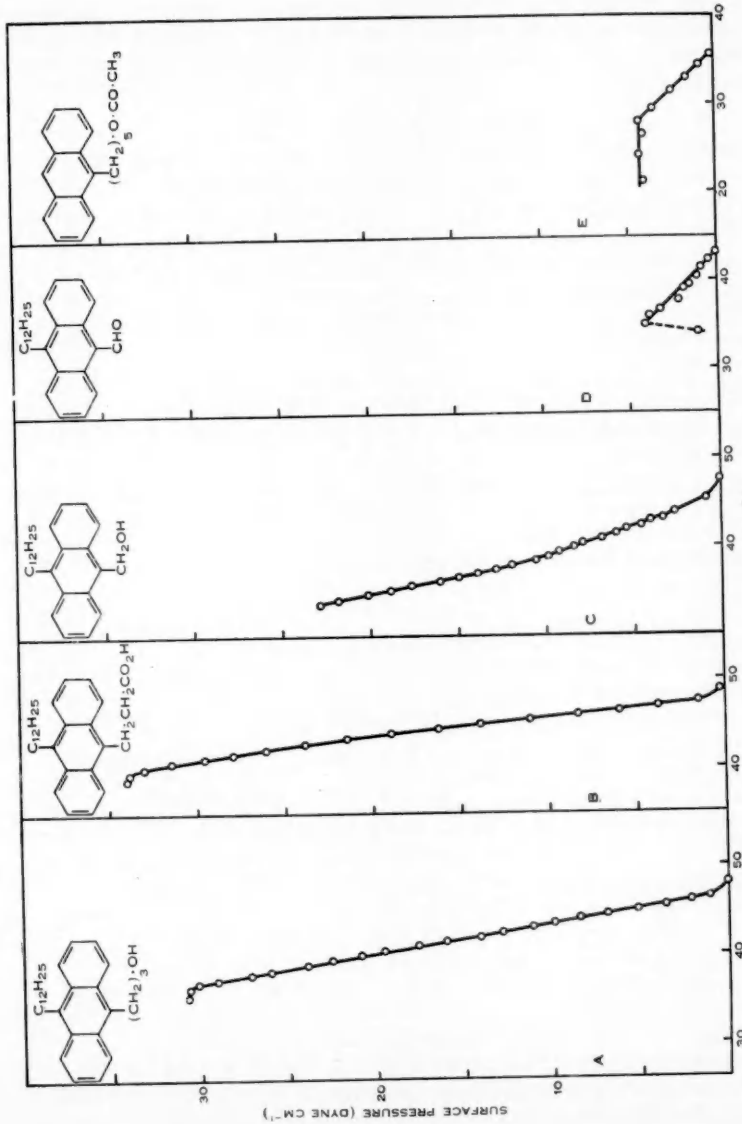

Fig. 2.—Surface pressure-area curves of several anthracene derivatives.

the molecule, 38 Å² along the long axis, and ~70 Å² in the plane of the ring system. The determination of the minimum molecular area in the compressed monolayer by inspection of models based on these dimensions must also take into account an important structural feature which is present in all these *meso*-anthracene derivatives, namely, a high degree of steric inhibition of rotation of the aliphatic side chains. This is easily seen from the models and is illustrated in Figure 3, which shows the extent of the van der Waals overlap which results when the side chains are rotated through the plane of the ring system. Some effects of steric hindrance on rotation in *meso*-substituted anthracene derivatives have been discussed by Trotter (1959).

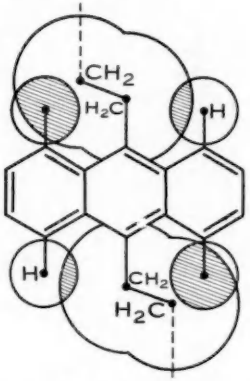

Fig. 3.—Scale drawing showing overlap of van der Waals radii in a planar model of a *meso*-dialkylanthracene.

This steric effect will tend to make the molecules adopt a strain-free configuration with the side chains projecting out of the plane of the anthracene nucleus. There are no quantitative values for the energy barrier opposing rotation in this particular system, but a value in the region of 10,000 cal for each side chain follows from a comparison with models of optically active diphenyls which owe their asymmetry to such restricted rotation, and for which the energy barrier has been calculated from the rates of racemization. It is probable that a compressed monolayer under this amount of internal strain would be very unstable, and that, consequently, the molecules will adopt the non-planar configuration in the stable monolayers of Table 1.

With these factors in mind the packing behaviour of both coplanar and non-coplanar models has been examined. The arrangement of strain-free molecules of 9-dodecyl-10-γ-hydroxypropylanthracene is illustrated in Figure 4,*

* In Figure 4, the water surface has been arbitrarily drawn through the terminal carbon atom of the hydrophilic side chain, but it is possible that the weak hydrophilic character of the anthracene nucleus facilitates greater immersion of the short side chain in the surface. In this connection Giles and Neustadter (1952) assumed that the aromatic nuclei in their long-chain azo compounds were either completely or partially immersed.

but the same conclusions should apply to the other compounds. When the planes of the anthracene nuclei are tilted towards the water surface as shown (Fig. 4 (*a*)) it is possible to pack the molecules into an area of some 45 Å^2 with the zigzags of the side chains dovetailing as in the close-packed monolayers of the long-chain aliphatic alcohols and acids. In this arrangement the long axis of the anthracene nucleus is parallel to the surface, but it is found that by pivoting the array of molecules about the line of anchorage in the surface a reduction of area to an optimum value of about 37 Å^2 takes place. This is shown in Figure 4 (*b*), and is due to the reduced magnitude of the projection in the surface of the now tilted long axis.

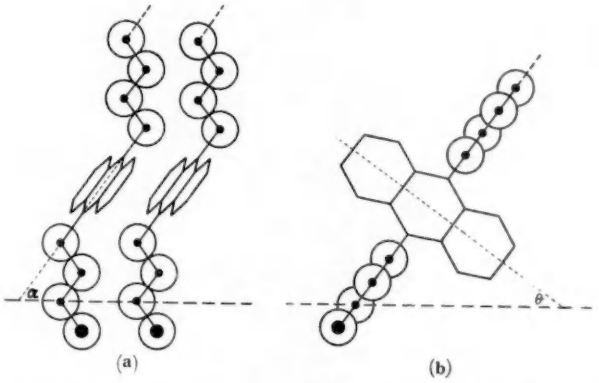

Fig. 4.—(*a*) Scale drawing to illustrate the close packing of molecules of 9-dodecyl-10-γ-hydroxypropylanthracene in a monolayer. Area per molecule, ~45 Å^2; $\alpha = 55°$. (*b*) The same basic arrangement, but tilted with respect to the vertical plane. Area per molecule ~37 Å^2; $\theta = 35°$.

These two areas correspond quite closely to the observed values of 46–48 and 35–39 Å^2 for the limiting area and collapse area, respectively, of the most stable films (Fig. 2), and suggest that the compressibility of the films is due to this tilting. The lower values observed for 9-ε-acetoxyamylanthracene (Fig. 2*E*) may be due to slightly closer packing resulting from the absence of a second *meso*-substituent in this compound. On the other hand, when the above steric factor is ignored and a planar model constructed (with necessary drastic bending and stretching of bonds) the molecules can now be accommodated in a minimum area of ~30 Å^2, with the plane of the anthracene nucleus perpendicular and the long axis tilted towards the surface.

These results indicate that steric inhibition of rotation in *meso*-substituted anthracene derivatives measurably influences the structure of their unimolecular films. It is possible that the smaller rotational barriers present in saturated carbon chains and in flexible alicyclic systems could also affect monolayer behaviour in certain circumstances, and that surface measurements might have some potential application in conformational studies with suitable compounds.

V. Acknowledgment

The author is indebted to Mr. W. W. Mansfield for helpful discussions during the course of this work.

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THE PROTON MAGNETIC RESONANCE SPECTRUM AND STRUCTURE OF HIMGRAVINE

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Summary

The high resolution proton magnetic resonance spectra of himgravine and himbacine have been obtained in $CHCl_3$ solution at 60 Mc/s. The spectra are consistent with only one of the two possible structures proposed. Other information with regard to relative orientation of groups within the molecule has also been obtained.

I. Introduction

Proton magnetic resonance has become a powerful tool for the solution of problems in structural organic chemistry (Pople, Schneider, and Bernstein 1959; Roberts 1959). The present investigation is a typical example of the use of p.m.r. in this field. It will be shown that only one out of two possible structures for himgravine is consistent with the p.m.r. spectrum. Other information with regard to configuration is also obtained from the magnetic resonance spectrum.

II. Materials and Methods

The magnetic resonance spectra of saturated solutions of himgravine and himbacine in chloroform were obtained at 60 Mc/s and are shown in Figure 1. The chemical shift data are in parts per million from the solvent peak, taking high field as positive. The intensity scale of the spectrum to the right-hand side of the dotted line is one-half of that to the left-hand side.

The samples of himgravine and himbacine were provided by Dr. E. Ritchie with the following information: Himgravine has structure (I) or (II) (Fig. 2) whereas in himbacine the 1 : 2 double bond is saturated (the numbering is shown in Fig. 2). As we shall see the p.m.r. spectrum of himgravine is sufficient to decide between these structures. However, the himbacine spectrum does provide a check on the assignments and is included.

III. Results and Discussion

Let us consider structures (I) and (II) (Fig. 2). The only protons in these structures, whose peaks would be expected to lie in the low field region (i.e. below 4·0 p.p.m.), are the ethylene protons on the $\alpha\beta$-carbon atoms, the proton on C_1 of structure (I), and the C_5 proton which is adjacent to an oxygen atom.

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There are three distinct sets of peaks in this region at 0·94, 2·0, and 3·3 p.p.m., with the intensity ratio of 1 : 2 : 1. This suggests that structure (I) is the correct structure as structure (II) would only give two low field groups. However, to confirm this it is necessary to assign the individual groups to the protons in structures (I) or (II). This can be done by considering the fine structure in each group.

The complex pattern centred at 2·0 can be analysed as an AB quartet (Bernstein, Pople, and Schneider 1957), each of the signals due to protons A and B being split further by interaction with one more proton (at $C_{6'}$ and C_7). This immediately assigns this group to the two protons of the $\alpha\beta$ double bond and the

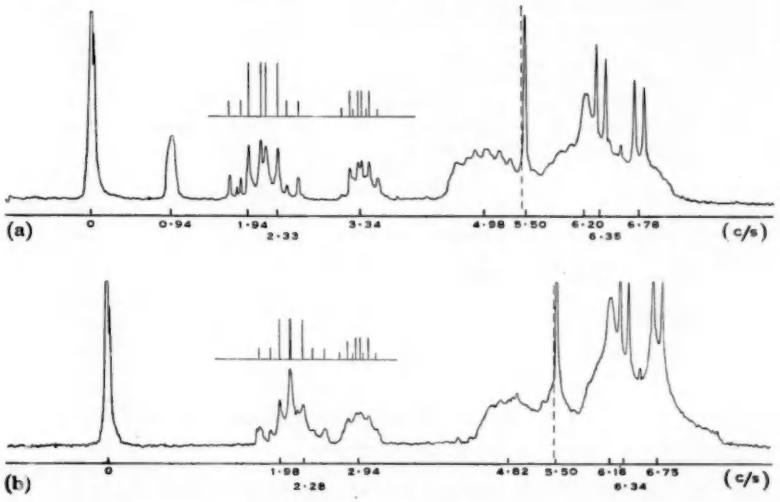

Fig. 1.—The proton magnetic resonance spectra of (*a*) himgravine and (*b*) himbacine. Chloroform is used as an internal reference.

coupling constants involved are $J_{\alpha\beta}$, $J_{\alpha 7}$, and $J_{\alpha 6'}$. (The coupling constants are the interactions between the protons on the numbered carbon atoms, e.g. $J_{\alpha 7}$ is the interaction between the proton on the α-carbon atom and that on C_7.) Although we know the values of these coupling constants we can only assign $J_{\alpha\beta}$ definitely as we do not know which peaks in the group are due to the proton on C_α and which are due to that on C_β.

The group of peaks at +3·34 can be seen to be about one-half the intensity of the above group and therefore is due to only one proton. The fine structure can be explained as a doublet split further by coupling with three equivalent protons. This group again has a unique assignment; it can only be due to the C_5 proton of structure (I) and the coupling constants involved here are J_{56} and $J_{5\text{-Me}}$. This assignment provides some evidence in favour of structure (I)

(structure (II) would give a simple quartet pattern for this hydrogen). However, the strongest support of structure (I) comes from the occurrence of the band at 0·94. It is of intensity one and can only be due to the C_1 proton of this structure. There is no possible assignment of this signal to any of the protons in structure (II). Thus we can immediately discard this structure. The fine structure of this band is not resolved. However, from the band width we can estimate the approximate size of the largest coupling constant involved, which can only be $J_{1\text{-}13}$. There appear to be in this case some small interactions with other protons, for example, J_{16}, which obscure the normal doublet fine structure. The calculated fine structure of the $C_{\alpha\beta}$ and C_5 groups is shown above the observed spectrum in Figures 1 (*a*) and 1 (*b*). The peak intensities have been normalized so that the intensity ratio of the two groups is 2 : 1.

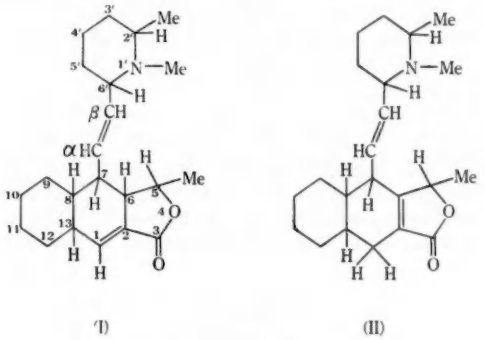

Fig. 2.—The possible structures for himgravine.

The assignments given above are confirmed by the spectrum of himbacine (Fig. 1 (*b*)). Again the intensity scale on the right-hand side of the dotted line is one-half of that to the left-hand side. We see that the groups at 2·0 and 2·9 have essentially the same fine structures as those in the himgravine spectrum and that the group at 0·9 is no longer present, as we would expect. Note that the appearance of the fine structure at 2·0 is slightly changed from that of the corresponding group in Figure 1 (*a*). This is due to a decrease in the chemical shift difference between the C_α and C_β protons on going from himgravine to himbacine. Chemical shift values are known to be very sensitive to small changes in the environment (e.g. note how the chemical shift of the C_5 proton has changed from 3·3 to 2·9) and thus this small change is not surprising.

By considering only the low field portion of the spectrum we have distinguished between the two possible structures of himgravine. We will now consider what additional information can be abstracted from the spectra. The high field part of both spectra is not very interesting. We can definitely assign the peaks at 5·5, 6·3, and 6·8 to the methyl groups on the nitrogen atom, on C_5 and on $C_{2'}$ respectively, and the doublet structure of the last two gives us the values of $J_{5\text{-Me}}$ and $J_{2'\text{-Me}}$. Of the remaining protons those near a double bond

or a carbonyl group or a nitrogen or oxygen atom would be expected to give peaks to lower fields than those of the normal CH_2 protons. The group of peaks at 4·98 can therefore be assigned to the protons on C_6, C_7, C_{13}, $C_{2'}$, and $C_{6'}$. However, this group is too unresolved and complex to permit any detailed analysis. The remaining protons merge into one broad peak centred at 6·2.

The complete assignment, with chemical shifts and spin coupling constants, is given in Table 1.

It is possible to derive some information about the relative configurations of some of the adjacent hydrogens in this molecule by considering the values of the coupling constants we have obtained.

TABLE 1

CHEMICAL SHIFTS AND COUPLING CONSTANTS IN HIMGRAVINE AND HIMBACINE

Proton	Chemical Shift (p.p.m. from $CHCl_3$)		Spin Coupling Constants for Both Himgravine and Himbacine (c/s)
	Himgravine	Himbacine	
H_1	0·94		
H_α	2·33	2·28	$J_{\alpha\beta}$ = 15·3
H_β	1·94	1·98	$J_{\alpha 7}$ = 8·9
H_5	3·34	2·94	$J_{\beta 6'}$ = 7·7
$H_{2'}$, $H_{6'}$, H_7, H_6, H_{13}	4·98	4·82	J_{56} = 9·0 J_{1-13} = 2–3
(CH_3)	6·20	6·16	$J_{5\text{-Me}}$ = 6·2
1′-Methyl	5·50	5·50	$J_{2'\text{-Me}}$ = 6·3
5-Methyl	6·35	6·34	
2′-Methyl	6·78	6·75	

The value of $J_{\alpha\beta}$ is characteristic of a *trans* coupling constant (Alexander 1958). Thus we can say quite definitely that the configuration at the double bond ($C_\alpha C_\beta$) is *trans*. Also the value of J_{56} is characteristic of a *trans*-configuration of the C_5 and C_6 protons (Lemieux *et al.* 1958). (The word *trans* in this context means exactly the same as in the double bond case, i.e. the two C—H bonds considered are at 180° to each other, looking along the C—C bond joining them.) It is possible to obtain a *trans*- (or nearly *trans*) configuration of these protons in whatever configuration the five- and six-membered rings are joined. If the ring junction is the *cis*-form, which is the less strained configuration, the C_5 and C_6 protons can only have an axial-axial configuration in order to give this large value of the coupling constant.

The remaining coupling constants which are of interest are J_{1-13}, $J_{\alpha 7}$, and $J_{\beta 6'}$. These are all interactions in the system $HC-\overset{H}{C}=C$. Alexander (1958) has measured the coupling constant in a similar system (butene-1) in which there is free rotation about the single bond. The value he obtained (6·3 c/s) represents the average of the coupling constants in the possible stereoisomers.

If we now consider the configuration of the protons on C_1 and C_{13}, the angle between these C—H bonds is about 60° (neglecting strain in the ring etc.) and thus we have from the observed band width that $J_{60°} \approx 2$–3 c/s. The value of $J = 6 \cdot 3$ from freely rotating butene-1 suggests that the *trans* coupling constant will be large (Pople, Schneider, and Bernstein 1959, p. 377 et seq.). This is in agreement with the results for saturated compounds.

We note that the values of $J_{\alpha 7}$ and $J_{\beta 6'}$ are significantly larger than the above value for the freely rotating case. This indicates that there is hindered rotation about the C—C bonds and that the *trans*-configuration is more favoured. On this basis we assign $J_{\alpha 7}$ as the larger of the two interactions as rotation about the C_α—C_7 bond would be expected to be more hindered.

In closing it is of interest to note that we cannot give any clue as to the configuration of the protons on C_6 and C_7, which would be of interest in determining the configuration of the ring junction, or of those on C_7 and C_8. However, a strongly electron attracting group on say C_9 may lower the chemical shift of C_8 proton into the low field region where it may be possible to analyse the fine structure.

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THE REACTION OF PROTEIN THIOL AND DISULPHIDE GROUPS WITH CUPRIC SULPHITE SOLUTIONS

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Summary

Thiol and disulphide groups in proteins react with neutral and alkaline cupric sulphite solutions and are thereby converted into thiolsulphate ($-SSO_3H$) groups. Solutions of cuprammonium sulphite at pH 9–10·5 can dissolve large amounts of keratins; under other conditions the disulphide bonds can be broken without the proteins entering solution. The reactions are highly specific, proceed to completion, and large excesses of reagent are not required. The "*S*-sulphokerateines" so obtained constitute a new class of water-soluble protein derivative. The thiolsulphate group can be readily labelled with radioactive sulphur and may be converted into a thiocyanate ("*S*-cyanokerateine") by reaction with cyanide, and into a mixed disulphide by reaction with a thiol. *S*-Cyanokerateines, containing the $-CH_2SCN$ side chain, react with cysteine to give thiocyanate ion and a protein containing lanthionine residues, presumably by nucleophilic displacement of SCN^- from the CH_2 group.

I. INTRODUCTION

It was shown by Kolthoff and Stricks (1951*a*) that cysteine (shown as RSH) reacts with cupric copper in ammoniacal solution in the presence of sulphite according to the equation

$$RS^- + 2Cu^{++} + SO_3^{--} \rightarrow RSSO_3^- + 2Cu^+, \qquad (1)$$

where $RSSO_3^-$ represents the anion of "*S*-sulphocysteine".† Cystine (RSSR) undergoes a nucleophilic displacement reaction with sulphite ion, to give an equilibrium mixture containing cysteine and *S*-sulphocysteine (eqn. (2)),

$$RSSR + SO_3^{--} \rightleftharpoons RS^- + RSSO_3^- \qquad (2)$$

so that in the presence of alkaline cupric copper, the overall reaction of cuprammonium sulphite with cystine is the sum of (1) and (2), namely,

$$RSSR + 2Cu^{++} + 2SO_3^{--} \rightarrow 2RSSO_3^- + 2Cu^+. \qquad (3)$$

Kolthoff and Stricks (1951*b*) used reactions (1) and (3) as the basis for analytical methods for cysteine and cystine. The compounds were titrated

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† The compound *S*-sulphocysteine, $HO_2C.CH(NH_2)CH_2.SSO_3H$ (2-amino-2-carboxyethanethiolsulphonic acid), has been known in the earlier literature as "cysteine sulphonic acid". The more rigorous name "*S*-sulphocysteine" has the particular advantage that it can be adapted in standard fashion when naming peptide sequences containing this amino acid residue. For example, the *S*-sulpho derivative of glutathione is γ-L-glutamyl-*S*-sulpho-L-cysteinylglycine. Similarly, when protein —SH and —SS— groups are converted into $-SSO_3H$, the products can be termed "*S*-sulphoproteins".

with cupric copper in ammoniacal sulphite solution and the appearance of cupric copper at the end-point was detected by polarography using a rotating platinum wire electrode (amperometric titration).

The present paper reports the application of reactions (1) and (3) to keratins and keratin derivatives, the —SH and —SS— groups being specifically and quantitatively converted into $-SSO_3^-$ groups. In subsequent papers, various extensions and developments of this method for breaking protein disulphide links will be reported. Preliminary accounts of the cupric sulphite method (Swan 1957, 1959) have led to use of the reaction in a variety of protein studies. McDermott and Pace (1959) have shown that a cuprammonium–sulphite–urea solution can be used to extract all the protein from wheaten flour, Pechère *et al.* (1958, 1959) have used a similar solution for splitting the disulphide bonds in trypsinogen and α-chymotrypsinogen while Weil and Seibles (1959) have used cuprammonium sulphite at pH 9 in the absence of urea to cleave all the disulphide bonds of α-lactalbumin and β-lactoglobulin.

Independently, Bailey (1957) has reported that a similar fission of disulphide bonds can be achieved by reaction with sulphite and an oxidizing agent such as iodosobenzoate or tetrathionate, and full details of this work have now appeared (Bailey and Cole 1959). Woods (1959) has reported electrophoretic studies on wool proteins solubilized by the cuprammonium sulphite procedure, and other physico-chemical studies are in progress in this laboratory.

II. Results and Discussion

(a) *Extent and Stoichiometry of the Reaction with Wool* (*Section III* (a), (b), *and* (f))

In preliminary experiments on the dissolution of keratins in cuprammonium sulphite it was found that wool could be partly dissolved (25–35%) by suspending samples for 24 hr in solutions containing a small excess of cupric ammonium hydroxide and 2–3 equivalents of sodium sulphite at pH 9·8–10·4. With longer reaction times (6–10 days), 70–80% of the wool was dissolved. In the presence of urea (8M), extraction was more rapid, 70–80% being dissolved after 24 hr, and this process was also applied successfully to horn and to feathers. When either the copper or the sulphite was omitted, virtually no protein was dissolved, even when urea was present. The *S*-sulphokerateines isolated from these cuprammonium sulphite extracts, and also the insoluble but chemically modified residues, were found not to contain either thiol or disulphide groups, suggesting that the reactions (1) and (3) proceed readily to completion under the various conditions used. In the work described, it is assumed that reactions (1) and (3) take the course shown, and that the extent of formation of *S*-sulphocysteine residues can be calculated from the amounts of thiol and disulphide remaining in the protein after treatment. Of particular value in this regard are the analytical methods of Leach (1959, 1960) which allow (SS + SH) to be measured on the intact protein, so that the problem of decomposition of *S*-sulphocysteine during hydrolysis in boiling 6N hydrochloric acid, to regenerate cysteine and cystine, is not encountered. When the residual (SS + SH) is zero (the usual result in the experiments reported) it is probably a reasonable assumption that

the reaction has gone to completion. When less than the theoretical amount of copper is used, intermediate stages in the reaction can be recognized (Kolthoff and Stricks 1951*a*; Leach and Swan, unpublished data) but no evidence has been obtained that such intermediates can persist in the presence of an excess of both sulphite and cupric copper. If the solution has access to oxygen, the cuprous copper can be continuously reoxidized so that the effective concentration of cupric copper remains high. In contrast, it may be noted that Bailey and Cole (1959), in their sulphite–iodosobenzoate method, used very large excesses of both reagents and treated the protein first with sulphite, then with the oxidant and repeated the cycle several times.

(*b*) *Cupriethylenediamine Sulphite Solutions* (*Section III* (c) *and* (d))

Cupriethylenediamine sulphite solutions were found to be rather less effective than cuprammonium sulphite solutions for the dissolving of wool, appreciable amounts of protein being dissolved only at pH values greater than 11. On the other hand, experiments with cupriethylenediamine sulphite/8M urea solutions showed that the extent of reaction (3) is not related to the amount of wool dissolved and indeed that the reaction can go to completion at pH values around 7 with scarcely any wool being dissolved at all.

(*c*) *Detection of "Alkali-Modification" of Wool* (*Section III* (d))

It was also shown that a cuprammonium–sulphite–urea mixture is a sensitive means for detecting "alkali modification" of wool. Wool, which had been heated at 50 °C at pH 11·1, was found to become less soluble with increasing time of heating, 82% being soluble initially and only 5% after pretreatment for 3 hr. The test mixture described here would seem to be more sensitive than the usual urea–bisulphite solution used for this purpose (Lees and Elsworth 1954, 1956, 1960).

(*d*) *Experiments on "Thiol-containing" Proteins and "Mixed Disulphide" Proteins* (*Section III* (e))

By reduction of wool with alkaline mercaptoacetate, kerateines containing large numbers of free SH groups can be prepared, and these thiol proteins could also be converted into *S*-sulphokerateines by reaction with cuprammonium sulphite (eqn. (1)). Removal of the excess mercaptoacetate was not necessary, since by using sufficient cuprammonium reagent, this thiol was also converted into its *S*-sulpho derivative. It was, however, more convenient to remove most of the excess mercaptoacetate by dialysis, in order to reduce the amount of cuprammonium sulphite required. During prolonged dialysis of kerateine–mercaptoacetate solutions, reoxidation occurs with formation of "kerateine–mercaptoacetate mixed disulphides"; these unsymmetrical disulphide bonds also react readily with cuprammonium sulphite. Thus the "dialysed kerateine" on polarography showed an anodic wave in the presence but not in the absence of sulphite (Fig. 1), which could be attributed to mercaptoacetate liberated from disulphide linkage with the protein by the action of sulphite (eqn. (4)).

$$\text{Ker.CH}_2\text{SSCH}_2\text{CO}_2^- + \text{SO}_3^= \rightleftharpoons \text{Ker.CH}_2\text{SSO}_3^- + {}^-\text{SCH}_2\text{COO}^-. \qquad (4)$$

No cathodic wave that could have been due to free $^{-}O_3SSCH_2COO^{-}$ was observed, so that it appears that sulphite fission of these mixed disulphides occurs exclusively in the sense of equation (4) and not at all in the alternative manner of equation (5):

$$Ker.CH_2SSCH_2CO_2^{-} + SO_3^{=} \rightleftharpoons Ker.CH_2S^{-} + {}^{-}O_3SSCH_2COO^{-}. \quad (5)$$

The same result was observed for kerateine-cysteine mixed disulphides prepared from *S*-sulphokerateines by dialysis against cysteine (see below); here again the action of sulphite liberated cysteine from mixed disulphide combination, but no polarographic evidence for liberation of *S*-sulphocysteine was obtained. This could mean either that the sulphur atom nearer to the protein chain is always more electrophilic than the "outer" sulphur atom, or that in these examples the low molecular weight thiol anion is a better "leaving group" in the nucleophilic displacement than is the protein thiol anion.

(*e*) *Isolation of* S-*Sulphokerateines* (*Section III* (f))

The preparation of copper-free *S*-sulphokerateines from cuprammonium sulphite extracts of keratins is discussed in Section III. In general, the standard techniques of dialysis, precipitation, chromatography, and lyophilization can be employed but the specific method used will naturally vary from one protein to another, and especially will depend on whether or not urea is present in the reaction mixture.

(*f*) *Specificity of the Reaction* (*Section III* (g))

To investigate the specificity of the reaction, various amino acids and peptides were treated both with sulphite alone and with cuprammonium sulphite. The formation of new ninhydrin-reacting spots, with partial or complete disappearance of the original spot, was noted only in the case of substances containing —SH or —SS— groups. For the reaction between thiols or disulphides and sulphite on filter paper, it was found that cupric ions were not required in order to bring about complete conversion into the *S*-sulpho compounds. Under these conditions—that is, when the sulphur compound is first spotted onto the paper, dried, and then treated with sulphite solution—the oxidant which brings about complete reaction in the sense of $RSSR \rightarrow 2RSSO_3H$ is presumably atmospheric oxygen, and the reaction is possibly catalysed by traces of metal ions present in the paper and reagents. Pechère *et al.* (1958, 1959) have also shown in the case of trypsinogen and α-chymotrypsinogen that the cuprammonium–sulphite–urea reagent is entirely specific to —SH and —SS— groups and that the reaction proceeds quantitatively. Weil and Seibles (1959) found that the reaction with α-lactalbumin and β-lactoglobulin was quantitative and highly specific. In these respects the method compares more than favourably with the peracid method for obtaining soluble protein derivatives by oxidation of —SH and —SS— groups to $-SO_3H$ (see Swan 1959).

(*g*) *Chemical Reactions of the* S-*Sulpho Group in Proteins* (*Section III* (h))

The chemical reactivity of the $-SSO_3^{-}$ group in *S*-sulphokerateines was investigated both by following the loss of radioactive sulphur from proteins containing $-S^{35}SO_3^{-}$ groups and also by amino acid and thiol/disulphide analysis,

and polarographic behaviour of the proteins formed under various conditions. Organic thiolsulphates, $RSSO_3^-$, are readily labelled with sulphur-35 by an exchange reaction with $^{35}SO_3^{--}$ (Fava and Pajaro 1956 ; Fava and Iliceto 1958 ; Milligan and Swan 1959) so that *S*-sulpho proteins can be labelled either during or subsequent to their preparation, and the label can similarly be removed by

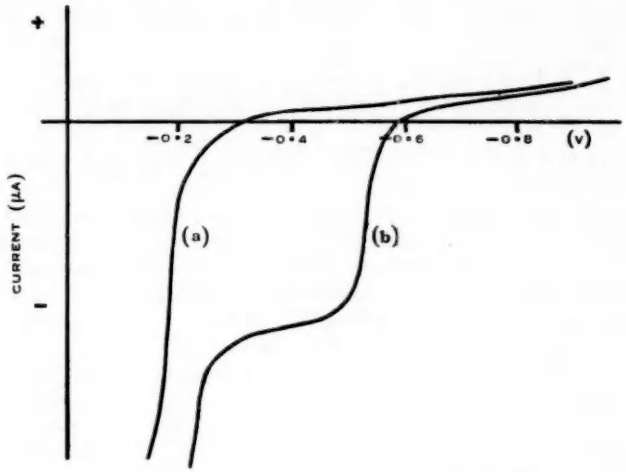

Fig. 1.—Polarograms of protein solutions. All solutions contained 8M urea, 0·5M KCl, 0·1M NH_4Cl, and NH_4OH to give a pH between 9·3 and 9·6. The type of curve observed in the presence and absence of 0·2M sulphite is indicated in the following :

Protein	Sulphite	Type of Curve
S-Sulphokerateine	Absent	(*a*)
	Present	(*a*)
A solution of kerateine containing mercaptoacetate which had been dialysed until —SH test was negative	Absent	(*a*)
	Present	(*b*)
S-Sulphokerateine after dialysis against either cysteine or mercaptoacetate and then water	Absent	(*a*)
	Present	(*b*)
S-Cyanokerateine after dialysis against cysteine and then water	Absent	(*a*)
	Present	(*b*)*

* Only a very small amount of cysteine was liberated.

dialysis against excess unlabelled sulphite (eqn. (6)) :

$$Ker.CH_2SSO_3^- + {}^{35}SO_3^{--} \rightleftharpoons Ker.CH_2S^{35}SO_3^- + SO_3^{--}. \qquad (6)$$

When labelled *S*-sulphokerateine was dialysed against a range of buffer solutions, pH 2·6 to 11·2, and also against cold 0·1N–6N hydrochloric acid, it was found that only slight losses of radioactivity occurred, and these small losses might well have been due to escape of protein through the dialysis membrane rather than to chemical decomposition of the $-SSO_3^-$ group. On the other

hand dialysis against 0·1M sodium hydroxide, pH 13, caused loss of all radioactivity, in accordance with the known decomposition of thiolsulphates in strong alkali (Price and Twiss 1910; Baumgarten 1930; Clarke 1932; Dornow 1939; Rosnati 1945). Simple alkyl thiolsulphates appear to be stable only within the pH range 3–9 at 50 °C (Fava and Pajaro 1956). Dialysis of the radioactive *S*-sulphokerateine against sulphite, cyanide, and thiols at pH values above 5, caused complete loss of all radioactivity from the protein according to equations (6), (7), and (8) respectively.

$$\text{Ker.CH}_2\text{S}^{35}\text{SO}_3^- + \text{CN}^- \rightarrow \text{Ker.CH}_2\text{SCN} + {}^{35}\text{SO}_3^{--} \quad (7)$$

$$\text{Ker.CH}_2\text{S}^{35}\text{SO}_3^- + \text{RS}^- \rightleftharpoons \text{Ker.CH}_2\text{SSR} + {}^{35}\text{SO}_3^{--} \quad (8)$$

$$\text{Ker.CH}_2\text{SSR} \xrightarrow{\text{excess RS}^-} \text{Ker.CH}_2\text{S}^- + \text{RSSR} \quad (9)$$

Equation (8) was confirmed by polarographic analysis of the products obtained by dialysing *S*-sulphokerateine against both cysteine and mercaptoacetate; Figure 1 shows the polarograms obtained from mixed disulphide proteins (Ker.CH_2SSR) in the presence and absence of sulphite.

It has been suggested (Swan 1957*b*; Parker and Kharasch 1959) that a likely mechanism for the formation of lanthionine (monosulphide) residues from cystine (disulphide) residues by the action of cyanide on proteins (eqn. (10)) is nucleophilic attack of CN^- on one of the sulphur atoms of the disulphide bond (eqn. (11)) followed by a second nucleophilic attack of the liberated thiol anion on the carbon atom holding the —SCN group, leading to ejection of thiocyanate ion by a conventional S_N2 process and formation of the monosulphide link (eqn. (12)). If the thiol anion attacks the sulphur atom of the —SCN group, the disulphide is simply reformed (reverse of eqn. (11)).

$$\text{Ker.CH}_2\text{—S—S—CH}_2\text{Ker.} + \text{CN}^- \longrightarrow \text{Ker.CH}_2\text{—S—CH}_2\text{Ker.} + \text{SCN}^- \quad (10)$$

$$\text{Ker.CH}_2\text{—S—S—CH}_2\text{Ker.} \rightleftharpoons \text{Ker.CH}_2\text{SCN} + {}^-\text{S—CH}_2\text{Ker.} \quad (11)$$

(+ CN^- attacks the sulphur bonded to the CH_2)

$$\text{Ker.CH}_2\text{—SCN} \longrightarrow \text{Ker.CH}_2\text{—S—CH}_2\text{Ker.} + \text{SCN}^- \quad (12)$$

(+ ${}^-S—CH_2Ker.$ attacks the CH_2)

When a sample of *S*-sulphokerateine was dialysed against cyanide, the *S*-cyanokerateine produced (eqn. (7)) was found to contain little or no disulphide; lanthionine also was present only in traces. However, when this *S*-cyanokerateine was dialysed against cysteine at pH 8·2, thiocyanate ions were liberated and the product was found to contain large amounts of lanthionine together with a much smaller amount of cystine. The cystine residues are probably formed by a nucleophilic displacement on sulphur (eqn. (13) cf. the right to left reaction of eqn. (11)), but the main reaction is presumably a nucleophilic displacement on

carbon, leading to lanthionine formation with liberation of thiocyanate as shown in equation (14) (cf. eqn. (12)):

$$\text{Ker.CH}_2\text{—S—CN} \;\underset{\text{SCH}_2\text{CH(NH}_2\text{)CO}_2\text{H}}{\longrightarrow}\; \text{Ker.CH}_2\text{SSCH}_2\text{CH(NH}_2\text{)CO}_2\text{H} + \text{CN}^- \qquad (13)$$

$$\text{Ker.CH}_2\text{—S—CN} \;\underset{\text{SCH}_2\text{CH(NH}_2\text{)CO}_2\text{H}}{\longrightarrow}\; \text{Ker.CH}_2\text{SCH}_2\text{CH(NH}_2\text{)CO}_2\text{H} + \text{SCN}^- \qquad (14)$$

The earlier suggestion (Cuthbertson and Phillips 1945) that lanthionine is formed by elimination of thiocyanic acid from the β-thiocyanoalanine residue, followed by addition of thiol to the acrylic double bond (eqn. (15)) would now appear to be less likely. In particular, no thiocyanate ion was liberated when the *S*-cyanokerateine was kept at pH 8·2 in the *absence* of cysteine, showing that the first step of the hypothetical equation (15) does not occur readily (cf. also Swan 1957*b*):

$$\text{CH—CH}_2\text{SCN} \rightarrow \text{C=CH}_2 \xrightarrow{\text{RSH}} \text{CH—CH}_2\text{SR} \qquad (15)$$
$$+\text{HSCN}$$

The cystine residues introduced into *S*-cyanokerateine by dialysis against cysteine (eqn. (13)) were shown to be present in " half-combined " form, that is, only the amino and carboxyl groups at one end of the cystine molecule are involved in peptide linkage. Thus polarography in the presence of sulphite revealed an anodic wave due to liberated cysteine (cf. Fig. 1) exactly as was found for the product obtained by dialysing *S*-sulphokerateine against cysteine (eqn. (8)).

It has been proposed recently by Zahn, Kunitz, and Hildebrand (1960) that a reaction analogous to that of equation (12) could explain the supposed formation of lanthionine residues during the action of sulphite on wool (eqn. (16)):

$$\text{Ker.CH}_2\text{—SSO}_3^- \;\underset{\text{S—CH}_2\text{Ker.}}{\longrightarrow}\; \text{Ker.CH}_2\text{—S—CH}_2\text{Ker.} + \text{S}_2\text{O}_3^{--} \qquad (16)$$

If this reaction were to proceed to any extent, it might be expected that reaction of cysteine with *S*-sulphokerateine would give rise to a protein containing at least some combined lanthionine. As reported here, hydrolysates of *S*-sulphokerateine were found to contain some lanthionine, but after dialysis of the same *S*-sulphokerateine against cysteine at pH 8·4 (which destroys all the $-SSO_3^-$ groups, see eqn. (8)), the protein hydrolysate was found not to contain even a trace of lanthionine. Hence it can be suggested that reaction (16) does not occur, at least not at pH 8·4 and 20 °C, and that the lanthionine which is found in hydrolysates of *S*-sulphokerateines and of sulphite-treated wools is an artefact produced from the *S*-sulphocysteinyl residues during the acid hydrolysis. This

result again emphasizes the importance of developing analytical methods for amino-acid residues in intact proteins, so that a preliminary acid hydrolysis is not required (Leach, Maclaren, and Swan 1960). Further support for the suggestion that the lanthionine is an " hydrolysis artefact " is found in the fact that the amount of lanthionine " produced " in wool by the action of bisulphite at 45 or 65 °C remains sensibly constant for times of heating ranging from 15 to 240 min (Zahn, Kunitz, and Hildebrand 1960). On the basis of equation (16), a progressive increase in lanthionine content with time of heating could have been expected.

The protein interconversions discussed in this paper are summarized in Figure 2.

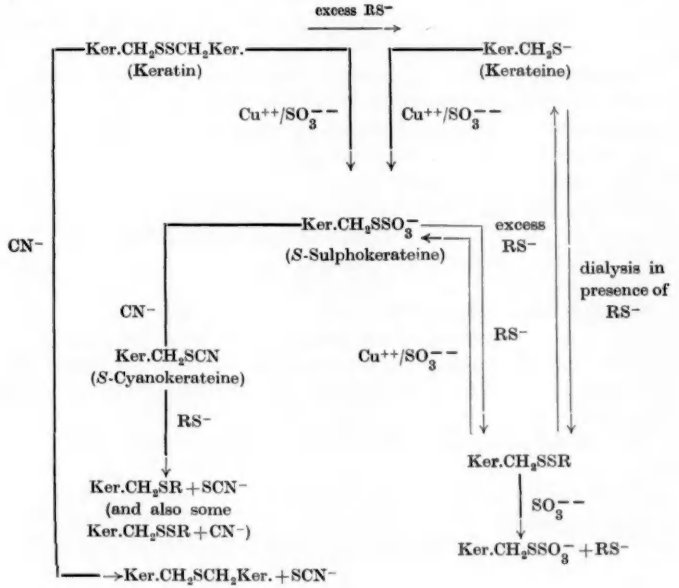

Fig. 2.—Summary of protein interconversions.
" Ker " signifies the keratin molecule other than the side chains of cysteine or cystine, and groups derived therefrom ; R = —CH_2CO_2H or —$CH_2CH(NH_2)CO_2H$.

The results establish that the cupric sulphite method for the fission of disulphide bonds is applicable to proteins, and also illustrate a number of special advantages of the process ; namely that the reaction is highly specific, it occurs under mild conditions in aqueous solution, symmetrical fission occurs, the " outer " sulphur atom of the —SSO_3H group can be readily labelled with sulphur-35, and finally, although the protein thiolsulphate group seems to be reasonably stable (and fully ionized) over the pH range 2–11, it can be readily transformed, if required, into other functional groups such as —SSR or —SCN.

III. Experimental

(a) *Materials, Methods, and Reagents*

The wool used in most experiments was a solvent scoured Merino 64's (code No. MW-52), moisture content 11·5%, —SS—, 480 μmole/dry g, —SH, 30 μmole/dry g. When stocks of this were exhausted a very similar wool, code No. MW-127, was employed. All reagents used were of A.R. quality. Sulphurous acid containing sulphur-35 (1·36 g of an aqueous solution containing 1·11% SO_2 w/w, specific activity initially 0·9 mc/g) was obtained from the Radiochemical Centre, Amersham. The solution was diluted to 25 ml with 1N NH_4OH and stored at —25 °C. Radioactivity in various protein solutions and dialysates was estimated as follows: Appropriate aliquots of the radioactive solution were neutralized to phenolphthalein using 10N NaOH and a further 2 ml was then added, followed by 30% H_2O_2 (2 ml). After 1 hr the solution was brought slowly to the boil and was acidified with concentrated HCl using bromphenol blue indicator. Sulphuric acid (5 ml; of 0·073N, ≡43 mg $BaSO_4$ after pptn.) was added, the solution was again brought to the boil and sulphate was precipitated with 2% $BaCl_2$ (3 ml). After standing for several hours or overnight the $BaSO_4$ was separated by centrifugation, washed with a little hot dilute HCl, and transferred in suspension in ethanol (5 ml) into a brass planchette, $\frac{3}{4}$ in. dia., $\frac{3}{16}$ in. deep. This planchette formed the base of a brass cylinder (1 in. high, $\frac{3}{4}$ in. dia.) from which it could be unscrewed when the $BaSO_4$ had settled out. In this way the bulk of the ethanol was run off over the top of the planchette, the remainder being removed by evaporation under an infrared lamp, leaving a uniform "infinitely thick" layer of $BaSO_4$. This sample was then counted using a thin-end Geiger-Müller tube (EHM 2[a]) with a conventional scalar unit.

Any residual disulphide in the soluble *S*-sulphokerateines or in the insoluble residues remaining after extraction was estimated by amperometric titration with either mercuric chloride or methylmercuric iodide in the presence of 0·2M sulphite, 8M urea, and appropriate buffer salts, according to the procedures developed by Leach (1959, 1960). A typical example is given. To an aliquot of *S*-sulphokerateine solution (20 ml) was added KCl (1·28 g), $Na_2SO_3.7H_2O$ (1·7 g), NH_4Cl (0·34 g), urea (16 g), and the pH of the solution was adjusted to 9·2–9·6 by addition of 17N NH_4OH. The final volume was 34 ml and a sample (20 ml) was then titrated with 10^{-2}M $HgCl_2$ at the dropping mercury electrode at a potential of —1·0 V *v.* a saturated calomel electrode. A polarogram from —0·2 to —1·5 V was recorded prior to the titration in order to observe whether any thiol of low mol. wt. was liberated from combination with the protein by the action of sulphite (evidence for mixed disulphide groups). If an anodic wave due to a low mol. wt. thiol was observed, a second polarogram was recorded in a mixture prepared similarly but with omission of the sodium sulphite. The absence of an anodic wave under these conditions was evidence that thiol was not present as an impurity in the solution.

The various cuprammonium and cupriethylenediamine solutions referred to in Sections (*b*) and (*c*) below were prepared as follows.

"1M cuprammonium hydroxide"—conc. NH_4OH (40 ml) was added to a solution of $CuSO_4.5H_2O$ (37·5 g) in water (90 ml) and the mixture diluted to 150 ml.

"0·08M cuprammonium hydroxide"—conc. NH_4OH (40 ml) was added to a solution of $CuSO_4.5H_2O$ (20 g) in water, and the mixture diluted to 1 l.

"0·08M cupriethylenediamine"—ethylenediamine (5–6 ml) was added to a solution of $CuSO_4.5H_2O$ (10 g) in water (500 ml) until the pH was 10·4.

Chromatography.—Lanthionine was determined qualitatively by two-dimensional chromatography on 20 cm square Whatman No. 1 papers using the fast running solvent mixtures of Hardy, Holland, and Nayler (1955). In solvent "E" (ethanol : 1-butanol : water : propionic acid = 10 : 10 : 5 : 2 by vol.), lanthionine, cystine, cysteic acid, and *S*-sulphocysteine all had R_F values close to 0·05, in solvent "O" (acetone : 1-butanol : water : dicyclohexylamine = 10 : 10 : 5 : 2 by vol.) the R_F values (on non-equilibrated paper) were respectively 0·36, 0·45, 0·50, and 0·60. If the papers were first equilibrated with solvent vapour before irrigation, all R_F values were increased by 30%. Hardy, Holland, and Nayler report R_F values of 0·61 for cystine and 0·49 for cysteic acid in solvent "O"; the reason for the reversal of the relative positions of cystine and cysteic acid on our chromatograms is not known.

(b) Dispersion of Keratins in Cuprammonium Sulphite Solutions

(i) To 0·1M $CuSO_4$ (10 ml) was added conc. NH_4OH (1 ml), 0·1M Na_2SO_3 (50 ml), and urea (29 g). Wool (0·23 g) was suspended in the solution and the mixture was shaken gently for 70 hr at 15 °C. The gelatinous residue was filtered off under suction on either hardened filter paper or glass filter cloth and was washed with 0·1M citrate buffer, first at pH 8, then at pH 6. The amount of wool taken into solution (82%) was calculated from the dry weight of this residue. When the experiment was repeated on a 50-fold scale, 84% was dissolved.

(ii) To 8M urea (1 l.) was added "1M cuprammonium hydroxide" (20 ml) and then 2M Na_2SO_3 (25 ml) giving a solution containing approx. 0·02M cuprammonium, 0·05M sulphite, and 8M urea, having pH 10·4. Wool (5 g) was shaken in this mixture for 24 hr at 22 °C in a closed flask, when 61% of the protein was dissolved. At a reaction temperature of 5 °C, 56% was dissolved after 24 hr, 63% after 22 days.

(iii) Using 8M urea (500 ml) and other quantities as in (ii) so that final concentrations were approx. 0·04M cuprammonium, 0·1M sulphite, 8M urea, pH 10·3, 60% of the protein was dissolved after 24 hr at 20 °C and 50% after 24 hr at 5 °C.

(iv) When water (500 ml) was used in place of the 8M urea of experiment (iii), final pH 9·8, only 33% of the protein had entered solution after 24 hr, but the insoluble material was, after washing and drying, largely soluble in 8M urea at pH 10.

(v) Wool (10 g) was suspended in a mixture of water (1 l.) "1M cuprammonium hydroxide" (40 ml), 2M Na_2SO_3 (50 ml), and 17N NH_4OH (10 ml), the pH being close to 10. After 4 days 83% of the wool was dissolved and after 8 days 89%. Similar solubilities were obtained when the volume of water was reduced by half, or was replaced by 8M urea (500 ml).

(vi) Urea (96 g) was dissolved in a mixture of "0·08M cuprammonium hydroxide" (50 ml), 2M Na_2SO_3 (50 ml), and sufficient water to give a final volume of 200 ml. The pH of this solution containing 8M urea, 0·02M cuprammonium, and 0·05M sulphite was 10·6. Wool samples (0·2 g) were shaken in aliquots (40 ml) of this mixture, in closed test tubes for 22 hr at 20 °C. The residues were filtered off, and were washed successively with 0·1M citrate buffer, pH 8·0, very dilute acetic acid, and water. Solubilities of 80–84% were obtained.

(vii) Whole adult fowl feathers were chopped to a coarse powder in a Wiley mill. Samples (0·25 g) were shaken with 8M urea (50 ml) containing "1M cuprammonium hydroxide" (1 ml) and 1M Na_2SO_3 (5 ml) for 64 hr at 20 °C. The material which remained undissolved (10%) was extremely swollen and gelatinous but could be readily filtered off after the whole mixture had been acidified to pH 5 with conc. HCl. In similar experiments, fowl feather rachis and powdered cow horn were dissolved in one extraction to the extent of 94 and 86% respectively.

(c) Dispersion of Wool in Cupriethylenediamine–Sulphite–Urea Solutions

(i) To "0·08M cupriethylenediamine" (100 ml) was added 0·2M Na_2SO_3 (100 ml) and urea (192 g), and the volume was adjusted to 400 ml, giving a solution containing 0·02M cupriethylenediamine, 0·05M sulphite, 8M urea, at pH 10·6. For tests on various wools, an aliquot (40 ml) was added to each sample (0·2 g) and the mixture was shaken gently for 22 hr at 20 °C. The control wool dissolved to the extent of 55–60%.

(ii) Aliquots (40 ml) of a solution of 0·02M cupriethylenediamine and 0·05M sulphite were adjusted to various pH values between 8·2 and 12·1 and to these were added wool samples (MW-127, 0·2 g) and the mixtures allowed to react for 48 hr at 20 °C. The amounts dissolved at pH values 12·1, 11·6, 10·3, 9·8, 9·2, and 8·2 were respectively 65, 12, —4, —1, —5, and —6%, the negative values indicating a weight gain.

(iii) Aliquots (40 ml) of a solution of 0·02M cupriethylenediamine, 0·05M sulphite, and 8M urea were adjusted to pH values between 12·2 and 5·9, and to these were added wool samples (MW-127, 0·2 g) and the mixtures allowed to react for 22 hr at 20 °C. The amounts dissolved at pH values 12·2, 11·7, 11·2, 10·7, 10·1, 9·4, 8·0, 7·0, and 5·9 were respectively 57, 26, 29, 22, 9, —1, —6, 1, and —5%. All the insoluble residues were washed thoroughly with water and dilute citric acid to remove excess copper and other reagents, and were then analysed for residual thiol plus disulphide by the amperometric methylmercuric iodide–sulphite–urea method (Leach 1960). Traces of copper left on the wool gave small polarographic waves which merged into the

larger wave due to excess methylmercuric iodide, so that the calculation of residual disulphide was subject to a small uncertainty. Nevertheless it was clear that in all the residues, including those from which scarcely any wool had dissolved, the amount of disulphide remaining was small and was probably zero.

(*d*) *Comparison of Sulphite Solutions Containing either Cuprammonium or Cupriethylenediamine for the Extraction of Chemically Modified Wool*

(i) Wool samples were subjected to mild alkali damage by heating them in pH 11·1, 0·2M borate buffer (wool to liquor ratio 1 : 100) at 50 °C for times 15, 30, 60, 120, and 180 min and were then washed successively in water, acetate buffer (pH 5·4), and water. Samples (0·2 g) were treated with cuprammonium–sulphite–urea and with cupriethylenediamine–sulphite–urea as described under (*b*) (vi) and (*c*) (i) respectively. Results are shown in Table 1.

TABLE 1

THE EFFECT OF MILD ALKALI PRETREATMENT ON THE SOLUBILITY OF WOOL IN CUPRAMMONIUM– AND CUPRIETHYLENEDIAMINE–SULPHITE–UREA SOLUTIONS

Time of Heating in pH 11·1 Buffer at 50 °C (min)	Solubility (%)*	
	In 0·02M Cuprammonium–0·05M Sulphite–8M Urea, pH 10·6, 22 hr, 20 °C, Wool : Liquor 1 : 200	In 0·02M Cupriethylenediamine–0·05M Sulphite–8M Urea, pH 10·6, 22 hr, 20 °C, Wool : Liquor 1 : 200
0	82	58
15	71	41
30	58	30
60	38	11
120	9	—0·4
180	5	—1·0

* Calc. on dry-weight basis ; a negative value indicates a weight gain.

(*e*) *Reaction of Reduced Wool Proteins ("Kerateines") and "Mixed Disulphide" Proteins with Cuprammonium Sulphite Solutions*

(i) A wool sample (2 g) was partly dissolved by heating it in 0·1M mercaptoacetate (pH 10·4 ; 200 ml) for 17 hr at 30 °C. The residue, representing about 40% of the keratin, failed to dissolve in a solution containing 8M urea and 0·08M sulphite at pH 9·5, but was more than 70% soluble in a similar solution containing also 0·2M cuprammonium hydroxide.

(ii) A wool sample (0·5 g) was heated for 17 hr at 50 °C in an aliquot (50 ml) of 0·35M sodium mercaptoacetate solution titrated to pH 11·0. The solution was filtered, the residue was washed with water (5 × 5 ml), and to the filtrate, representing 75% of the wool, and still containing a large excess of mercaptoacetate, was added 2M Na_2SO_3 (50 ml) and cuprammonium hydroxide prepared from 1M $CuSO_4$ (50 ml) and conc. NH_4OH (15 ml). There was a rapid transitory formation of a black precipitate, which redissolved to give a clear deep blue solution. After 5 min this solution was dialysed against 0·2M ammonium citrate, pH 8·7. With frequent changes over several days a copper-free protein solution was finally obtained. Amperometric titration showed SS and SH groups to be absent. An aliquot of this *S*-sulphokerateine solution was dialysed for 24 hr against 0·1M mercaptoacetate, pH 9·5, and then for several days against water, yielding a protein which contained at least some kerateine-mercaptoacetate mixed disulphide groups (see Section III (*h*) (ii) below) and which gave a positive $HgCl_2$ titre in the presence, but not the absence, of sulphite. Application of the cuprammonium sulphite reaction to this "mixed disulphide" protein again yielded an *S*-sulphokerateine for which the $HgCl_2$ titre in the presence of sulphite was zero.

(iii) A kerateine solution, prepared as described in (ii), was dialysed for several days against water until the nitroprusside test for SH was negative. Polarography in the presence and absence of sulphite (see Fig. 1 and Section (*h*) (ii) below) showed that sulphite caused liberation of mercaptoacetate from the protein: in this latter case the $HgCl_2$ titre was positive. Treatment of the protein solution with cuprammonium sulphite, followed by dialysis, gave an *S*-sulphokerateine for which the $HgCl_2$ titre was again zero.

(*f*) *Isolation and Analysis of* S-*Sulphokerateines*

In the case of wool proteins dissolved by cuprammonium sulphite in the absence of urea, the bulk of the *S*-sulphokerateine could be precipitated by acidifying to pH 3–4 with HCl, the addition of some $(NH_4)_2SO_4$ rendered the proteins even less soluble. The product still contained adsorbed copper, which was removed by dialysis against citrate or ethylenediaminetetra-acetate buffers at pH 7–9. Cuprammonium extracts containing urea were dialysed, first against ammonium citrate buffer (pH 8–9), to remove the bulk of the copper and the urea, and then against water or other appropriate buffer solutions. The extracts could not be dialysed directly against water as this led to some precipitation of cupric and cuprous hydroxides with clogging of the dialysis membrane. Protein was recovered from the dialysed solutions by freeze drying, or else by precipitation with acid or ammonium sulphate after concentrating the solution by perfusive evaporation through Cellophane.

With cupriethylenediamine–sulphite–urea extracts it was possible to dialyse directly against water since the cupriethylenediamine complex is soluble over the whole pH range. However, to remove the last traces of copper it was still necessary to dialyse against a complexing agent such as citrate, ethylenediaminetetra-acetate, or aqueous ethylenediamine.*

Various samples of *S*-sulphokerateines, prepared from wool, from feathers, and from both soluble and insoluble thiol-rich kerateines, were all shown by amperometric $HgCl_2$ titration not to contain any residual SH or SS groups.

(*g*) *Specificity of the Alkaline Cupric Sulphite Reaction*

The possibility of reaction between the cuprammonium sulphite reagent and amino acids other than cystine and cysteine was investigated chromatographically. Each substance to be tested (2–4 mg) was dissolved in water or dilute acid and three separate spots (5 μl) were applied to Whatman No. 1 paper. After drying, one spot was treated with 0·2M sulphite (5 μl), the second with a solution containing 0·2M sulphite and 0·1M cuprammonium hydroxide (5 μl). The chromatograms were then developed in 75% phenol in an atmosphere containing NH_3 and HCN and the amino acids were detected by the usual ninhydrin spray. All the common amino acids were tested and also *S*-benzylcysteine, cysteic acid, lanthionine, 3,5-dichlorotyrosine, *S*-2′-aminoethylcysteine, ornithine, methionine *SS*-dioxide, citrulline, α-aminoisobutyric acid, glycinamide, and the peptides glycylglycine, leucylvaline, glutaminylasparagine, carnosine, alanylmethionine, and ε-benzyloxycarbonyl-lysylglycine ethyl ester. None of these substances was affected by sulphite or by cuprammonium sulphite as judged by the intensity of the ninhydrin colours and the absence of new spots on the chromatograms. On the other hand cystine, cysteine, glutathione, bisglycylcystine, cystylbisglycine, and bisglycylcystylbisglycine, having R_F values of 0·33, 0·33, 0·40, 0·50, 0·45, and 0·62 respectively, were replaced by new spots of much lower R_F value (0·09, 0·09, 0·09, 0·13, 0·16, and 0·20 respectively), corresponding to conversion into the *S*-sulpho derivative. Reaction to give the *S*-sulpho compound as sole product was effected both by cuprammonium sulphite and by sulphite alone. In the latter case, the oxidant is presumably air.

(*h*) *Chemical Reactions of the* S-*Sulpho Group in Proteins*

(i) *Experiments Using Radioactive Sulphite.*—Wool (0·24 g) was shaken for 24 hr at 20 °C in a mixture containing 0·01M cuprammonium hydroxide (8 ml), 0·1M Na_2SO_3 (16 ml), urea

* Mr. E. F. Woods has shown recently in this laboratory that the bulk of the copper can be removed from cuprammonium-sulphite solutions of wool proteins by passing the solution down a column of Dowex-50 resin in the ammonium salt form, when the copper is retained on the resin.

(18 g), and the radioactive sulphite solution (4 ml). The solution was acidified with 10N HCl to pH 3–4 and the small amount of residue was filtered off. On diluting with an equal volume of water some *S*-sulphokerateine precipitated, and was collected, dissolved in 0·1N $KHCO_3$ (40 ml; pH 7·9) and dialysed at 5 °C against 11 successive changes (each 230 ml) of 0·1N $KHCO_3$. The total radioactivity in each of these dialysates, counted as $BaSO_4$ as described in (*a*) above, fell from 1300 c.p.m. for the first to 122, 97, and 92 c.p.m. for the last three solutions. An aliquot (1 ml) of the dialysed protein solution (total volume 42 ml) gave $BaSO_4$ = 2680 c.p.m., so that the radioactivity lost in the 11th dialysate represented approximately 0·08% of the total remaining inside the bag. Aliquots (1 ml) of the protein solution were then diluted with an equal volume of water and dialysed at 5 °C for 43 hr against aliquots (40 ml) of the solutions shown in the left-hand side of Table 2. Radioactivities in both the dialysates and the protein solutions were then measured and the percentages of total radioactivity liberated by the protein are also shown in Table 2. In a second experiment, aliquots (1 ml) of the dialysed radioactive *S*-sulphokerateine solution were dialysed for a further 18 hr against 0·1M $KHCO_3$ and then at 5 °C for 43 hr against 40 ml portions of the reagents shown in the right-hand side of Table 2. Results are given as before.

TABLE 2

LOSS OF RADIOACTIVE SULPHUR FROM *S*-SULPHOKERATEINE CONTAINING $-S^{35}SO_3^-$, ON DIALYSIS AGAINST VARIOUS SOLUTIONS FOR 43 HR AT 5 °C

Dialysis Solution	Loss of Radioactivity from Protein (as % of total present)	Dialysis Solution	Loss of Radioactivity from Protein (as % of total present)
0·1M Acetic acid, pH 2·6 ..	0·9	6M HCl	6·0
0·2M Acetate buffer, pH 4·3	1·7	1M HCl	1·8
0·2M Acetate buffer, pH 5·1	1·9	0·1M HCl	0·9
0·1M $KHCO_3$, pH 7·9 ..	1·7	0·1M Acetic acid containing 0·2% mercaptoacetic acid, pH 2·5	8·4
0·1M Citrate buffer, pH 8·8	3·2		
0·1M NH_4OH, pH 10·9 ..	7·3		
0·1M Na_2CO_3, pH 11·2 ..	7·8	0·1M Acetate buffer, saturated with H_2S, pH 5·3 ..	99
0·1M NaOH, pH 13	96		
0·1M $KHCO_3$, 1% KCN ..	98	0·1M $KHCO_3$ containing 0·2% mercaptoacetic acid, pH 7·4	99
0·1M $KHCO_3$, 0·1M Na_2SO_3, pH 8·6	95	0·1M $KHCO_3$ containing 0·2% toluene-ω-thiol, pH 8·6 ..	99
		0·1M NH_4OH, pH 10·6 ..	4·7

(ii) *The Reaction of* S-*Sulphokerateines with Thiols.*—A solution of *S*-sulphokerateine was dialysed for 24 hr against 0·1M mercaptoacetate, pH 9·5, and then for several days against running water. A polarogram recorded for a solution containing also urea, sulphite, chloride, etc. pH 9·4 (see Section (*a*)) showed an anodic wave characteristic for mercaptoacetate ($E_{\frac{1}{2}}$ —0·5 V) and a positive titre of $HgCl_2$ was obtained. In the absence of sulphite, no anodic wave was observed (cf. Fig. 1). In a similar experiment, a 2·8% solution of *S*-sulphokerateine (50 ml) was dialysed for 24 hr against a 0·05M cysteine hydrochloride–0·1M sodium borate solution, pH 8·4 (500 ml), and was then dialysed for 3 days against many changes of distilled water. The volume of the solution rose to 66 ml, the protein concentration fell to 1·6%. The initial *S*-sulphokerateine solution gave a zero $HgCl_2$ titre in the presence of sulphite and urea; acid-hydrolysis of the protein gave rise to cystine and a trace of lanthionine. After dialysis against cysteine, the protein then showed an anodic cysteine wave ($E_{\frac{1}{2}}$ —0·5 V) in the urea–sulphite mixture (Fig. 1); $HgCl_2$ titration showed that the SH+SS content had risen from zero to 417 μmole/g protein.

In the absence of sulphite, no cysteine wave was recorded on the polarogram, and $HgCl_2$ titration under these conditions gave a value of 56 μmole SH/g, assuming a stoichiometry of one $HgCl_2$ for each two SH groups. An acid hydrolysate of this " mixed disulphide " protein was found to contain much cystine, but lanthionine was now absent.

(iii) *Preparation and Properties of* S-*Cyanokerateines.*—In preliminary experiments on the conversion of $-SSO_3^-$ into —SCN (see also (i) above), solutions of *S*-sulphokerateine were treated with KCN at pH values ranging from 7·9 to 11·9. No thiocyanate ions were detected in these solutions after keeping for 24 hr, but positive tests were obtained after short boiling of the solutions and also after treatment in the cold with cysteine. To study this reaction of *S* cyanokerateine with cysteine, a solution of *S*-sulphokerateine, which had been thoroughly dialysed against 0·1M citrate buffer, pH 8·5, and which gave a zero $HgCl_2$ titre in the presence of sulphite and urea, was dialysed for 24 hr at 5 °C against 0·05M KCN, 0·05M sodium borate, pH 9·3, and finally against water for 4 days to remove all excess cyanide ions. Amperometric titration then revealed the presence of a small amount (62 μmole/g) of disulphide in this *S*-cyanokerateine. An aliquot (10 ml) was mixed with an equal volume of 10N HCl and heated under reflux for 5 hr. Two dimensional chromatography showed the presence of some cystine, with traces only of cysteic acid and lanthionine. Another aliquot of the *S*-cyanokerateine was then dialysed for 16 hr against a 10-fold volume of 0·025M cysteine hydrochloride solution, brought to pH 8·2 with sodium borate, and was then dialysed exhaustively against distilled water. Thiocyanate was liberated into the cysteine solution and amperometric titration of the dialysed protein showed that the SS content had increased somewhat to 170 μmole/g. The polarogram in the presence of sulphite showed an anodic cysteine wave (cf. Fig. 1). After hydrolysis, chromatography showed the presence of a small amount of cystine and a very strong spot due to lanthionine. In a second experiment, the solution of *S*-cyanokerateine gave no lanthionine on hydrolysis, but after dialysis against cysteine and then water, the hydrolysate was again shown to contain a large amount of lanthionine. In this case neither the *S*-cyanokerateine nor the product obtained therefrom by reaction with cysteine contained SS groups.

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SIMPLIFIED ANALOGUES OF LYSERGIC ACID

IV. SYNTHESIS OF 1-METHYL-1,2,3,7,8,9-HEXAHYDRO-5,6-BENZQUINOLINE

By J. CYMERMAN CRAIG,* DIANA M. TEMPLE,* and (in part) B. MOORE*

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Summary

The lysergic acid analogue 1-methyl-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (III; R=H) was prepared from 2-bromo-α-tetralone by two parallel routes. The first, a seven-stage sequence, involved conversion of the starting material into 2-[*N*-methyl-*N*-(2′-oxo-n-propyl)]-amino-α-tetralone (IX) via its ethylene ketal, followed by ring closure to the tricyclic 1-methyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (V). This was transformed into its ethylene dithioketal (XXIX) and thence by desulphurization into (III; R=H).

The second pathway, a five-step synthesis, utilized dimethylaminoacetone to obtain directly the quaternary diketone (XXI), which was cyclized to 1,1-dimethyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinolinium bromide (XXV), identical with that obtained by the alternative route.

I. INTRODUCTION

The marked pharmacological activity found in *N*-alkylated derivatives of 1,2,3,4-tetrahydro-2-naphthylamine (I), particularly in the *N*-methyl-*N*-n-propyl compound (II) (Cymerman Craig, Moore, and Ritchie 1959) which exhibited high and specific anti-serotonin activity (Pennefather and Thorp 1958) and hypotensive action (Pennefather and Thorp 1959), makes of considerable interest the preparation of 1-methyl-1,2,3,7,8,9-hexahydro-5,6-benzquinoline-3-carboxylic acid (III; R=COOH) and of the corresponding base (III; R=H) constituting the structure of rings A, C, and D of the lysergic acid molecule (IV), but lacking ring B. These syntheses have been examined, and considerable progress made. In view of a recent publication (Leemann and Fabbri 1959), our results leading to the preparation of 1-methyl-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (III; R=H) are now reported.

The synthesis of the tricyclic conjugated ketone 1-methyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (V) was carried out by two pathways. Bromination of α-tetralone by a modification of the method of Wilds (1942) and Wilds and Johnson (1946) gave 2-bromo-α-tetralone (VI; $R_1=R_2=H$) showing ultraviolet absorption similar to that of α-tetralone (Table 1). Hassner and Cromwell (1958*a*) have shown that the bromine in 2-bromo-4,4-dimethyl-α-tetralone (VI; $R_1=R_2=Me$) occupies the equatorial position ($\nu_{max.}$ 1705 cm^{-1}), while it is axial in both 2-benzyl-2-bromo- and 2-bromo-2-(α-bromobenzyl)-4,4-dimethyl-α-tetralone ($\nu_{max.}$ 1692 and 1690 cm^{-1} respectively).

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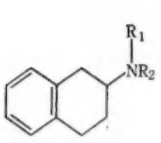
It is known that an equatorial bromine adjacent to the C=O of cyclohexanones effects a displacement of +16 to +22 cm^{-1} in the carbonyl frequency (Jones *et al.* 1952), while an axial bromine, which causes only a slight shift in the C=O region, will result in a bathochromic displacement of 10 to 15 mμ in the high-intensity (K) band of the ultraviolet spectrum (Cookson 1954). Moreover, equatorial carbon-bromine linkages in steroids have been shown to absorb at 703–772 cm^{-1}, and the corresponding axial linkages at 591–692 cm^{-1} (Barton, Page, and Shoppee 1956).



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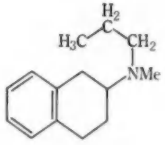

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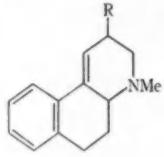

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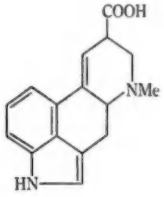

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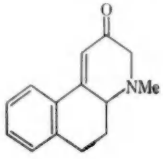

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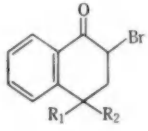

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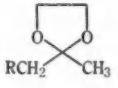

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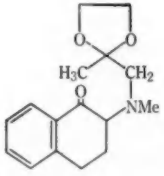

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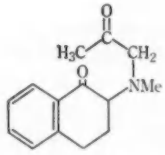


(IX)

Since the infrared absorption of 2-bromo-α-tetralone ($\nu_{max.}$ 1685, 756, 730, and 642 cm^{-1}) was identical with that of α-tetralone ($\nu_{max.}$ 1685, 761, and 731 cm^{-1}), the bromine in the former compound appears to be in the axial position, as confirmed by the bathochromic shift (+7 mμ) in the ultraviolet. The absorption band at 642 cm^{-1}, in a region characteristic of an axial carbon-bromine bond, is further evidence for the axial conformation of the halogen atom in 2-bromo-α-tetralone.

TABLE 1

ULTRAVIOLET LIGHT ABSORPTION IN 95% ETHANOL

Compound	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$
α-Tetralone	249 294	11800 1800
2-Bromo α-tetralone (VI; $R_1=R_2=H$)	256 297	10400 3000
4,4-Dimethyl-α-tetralone*	246 289	10900 1500
2-Bromo-4,4-dimethyl-α-tetralone (VI; $R_1=R_2=Me$)*	251 294	11200 1800
2-Benzyl-4,4-dimethyl-α-tetralone*	248 300	12600 1700
2-Benzyl-2-bromo-4,4-dimethyl-α-tetralone*	258 292	10600 2500
2-Benzyl-α-tetralone†	247 292	12600 1700
2-[*N*-Methyl-*N*-(2′-oxo-n-propyl)]amino-α-tetralone 2′-ethylene ketal (VIII)	247 295	14000 2400
2-[*N*-Methyl-*N*-(2′-oxo-n-propyl)]amino-α-tetralone (IX)	250 298	15000 1800
1-Methyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (V)	228 233‡ 298	8600 7800 14200
1-Oxo-1,2,3,4,9,10-hexahydrophenanthrene (XII)‖	230 236 298	13000 13000 17500
3,3a,4,5-Tetrahydrobenz[e]inden-2-one (XIII)‖	223·5 287	12000 24000
Benzalacetone (XIV)‖	220·5 286	12000 23500
Hydrobromide of (V)	228 235‡ 295	10800 9500 20300
3-Hydroxy-1-methyl-7,8-dihydro-5,6-benzquinolinium hydroxide betaine (XVIII)	251 355	13900 5300

TABLE 1 (*Continued*)

Compound	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$
4-Acetyl-4,5,5a,6-tetrahydro-9-hydroxy-7-methylindolo(4,3,-fg)-quinolinium hydroxide betaine (XX)§	246 351	29000 6900
NN-Dimethyl-*N*-(α-oxo-2-tetralyl)-*N*-(2′-oxo-n-propyl)ammonium bromide (XXI ; X=Br)	234 255 295	14800 7050 5050
1,1-Dimethyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinolinium bromide (XXV ; X=Br)	231 265 310	8600 9600 19300
1,3-Dimethyl-3-hydroxy-5,6-(1′,2′-naphtho)-2,3-dihydro-1,4-oxazine (XV)	248 340	13600 6100
1,1-Dimethyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinolinium bromide ethylene dithioketal (XXVIII ; X=Br)	281 305	8500 4100
1-Methyl-1,2,3,7,8,9-hexahydro-5,6-benzquinoline picrate (III ; picrate)	250 304 360	24600 12400 20700

* Hassner and Cromwell (1958*a*).
† Hassner, Cromwell, and Davis (1957).
‡ Inflexion.
|| Wilds *et al.* (1947).
§ Kornfeld *et al.* (1956).

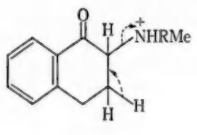
The first route for the preparation of the tricyclic ketone (V) was similar to that used by Kornfeld *et al.* (1956) for the synthesis of lysergic acid. The method of Kornfeld *et al.* (1956) for the reaction of bromoacetone ethylene ketal (Kuhn 1940) (VII ; R=Br) with anhydrous methylamine gave low yields in small-scale preparations, but alcoholic methylamine afforded good conversion to the desired methylaminoacetone ethylene ketal (VII ; R=CH_3NH). Reaction of this with 2-bromo-α-tetralone was complete after 6·5 hr and gave the ketal-ketone (VIII), showing infrared absorption at 1687 cm^{-1} as in 2-benzyl-α-tetralone ($\nu_{max.}$ 1686 cm^{-1}) (Hassner, Cromwell, and Davis 1957) and ultraviolet absorption similar to this compound (Table 1).

No ketonic derivatives of the ketal-ketone (VIII) could be prepared ; the substance was characterized as the picrate and hydrobromide. Leemann and Fabbri (1959) obtained this base from the same reactants after refluxing for 20 hr.

Removal of the protecting ketal group was effected by 3N hydrochloric acid, to give the unstable diketone (IX) as a yellow solid which rapidly darkened on exposure to air. To obtain this compound, it was found essential to conduct all operations under nitrogen and at the lowest possible temperatures, and to use peroxide-free solvents. It is noteworthy that Kornfeld *et al.* (1956) refer to the

corresponding diketone obtained in their lysergic acid synthesis, as "very susceptible to aerial oxidation".

In presence of air, the hydrolysis of the ketal-ketone (VIII) led only to α-naphthol, probably arising by an intramolecular elimination of tertiary amine from the protonated 2-amino-α-tetralone (X) to give the dienone (XI) which would undergo a facile rearrangement to the resonance-stabilized α-naphthol.



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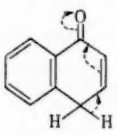

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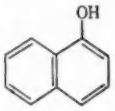

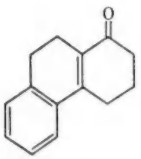

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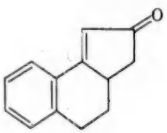

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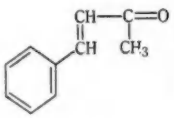

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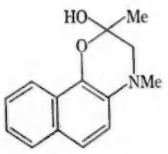

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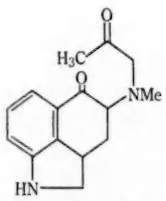

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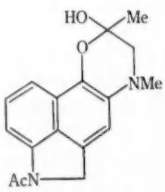
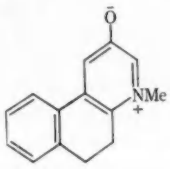


(XVII)

Again, no carbonyl derivatives could be prepared from (VIII), but a hydrobromide and a methiodide were obtained. The infrared absorption maxima of (IX) confirmed the presence of aliphatic (1710 cm^{-1}) and aromatic (1690 cm^{-1}) carbonyl functions, and its ultraviolet spectrum (Table 1) resembled that of its ketal-ketone precursor (VIII).

Cyclization of the diketone (IX) was achieved using sodium methoxide or potassium t-butoxide at low temperatures, affording the unstable tricyclic ketone (V). This showed ultraviolet absorption at 228, 233 (inflex.), and 298 mμ (Table 1), resembling that of the similar chromophoric systems in the phenanthrene derivative (XII) and the benzindene (XIII), and also in the open-chain analogue benzalacetone (XIV) (Table 1).

Cyclization of (IX) with 50% sulphuric acid at 0 °C (Adamson *et al.* 1937; Hills and McQuillin 1953) also resulted in a product identical with (V) in its light absorption properties. Its infrared spectrum demonstrated the disappearance of the aliphatic carbonyl group at 1710 cm^{-1}, and the shift of the aromatic carbonyl absorption from 1690 to 1665 cm^{-1} indicated the introduction of a conjugated double bond between the carbonyl group and the benzene ring



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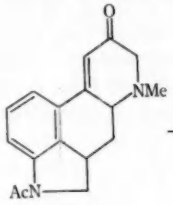

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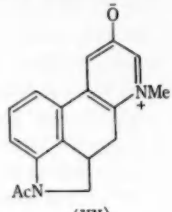

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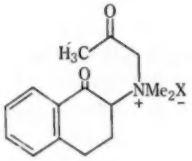

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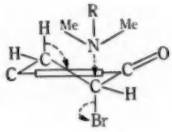

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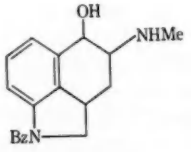

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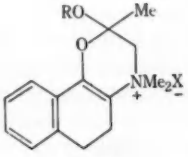


(XXIV)

in (V). The base was characterized as its hydrobromide and methiodide. Leemann and Fabbri (1959) were able to obtain the ketone (V) only by direct action of polyphosphoric acid on the ketal-ketone (VIII), and failed to achieve ring-closure under alkaline conditions.

An unsuccessful attempted cyclization of the diketone (IX) gave, on treatment with dry hydrogen chloride, a product which showed absorption at 3300 cm^{-1} (OH) but none in the 1650–1750 cm^{-1} region, and must therefore be the hydrochloride of the cyclic hemiketal (XV) formed by air oxidation of (IX) under acidic conditions. Its analysis and ultraviolet spectrum are in agreement with this aromatic structure. Kornfeld *et al.* (1956) obtained the closely related hemiketal (XVII) by treatment of (XVI) with acetic anhydride in methanol.

A water-soluble by-product obtained from the alkaline cyclization of (IX) showed ultraviolet absorption ($\lambda_{max.}$ 251 and 355 mμ) resembling that of a benzquinoline, and on the basis of its analysis, solubility, and light absorption must be the stable 3-hydroxy-1-methyl-7,8-dihydro-5,6-benzquinolinium hydroxide betaine (XVIII) formed by air oxidation of (V) in alkaline solution. The closely related betaine (XX) obtained by Kornfeld *et al.* (1956) from the tetracyclic ketone (XIX), absorbed at 246 and 351 mμ (Table 1).

The second synthetic pathway leading to the tricyclic ketone (V) involved the formation of the intermediate quaternary ammonium salt (XXI), prepared by reaction of dimethylaminoacetone with 2-bromo-α-tetralone.

A synthesis of dimethylaminoacetone from chloroacetone and aqueous dimethylamine (Stoermer and Dzimski 1895) gave low yields. However, the reaction of liquid dimethylamine and bromoacetone ethylene ketal proceeded smoothly in an autoclave to give the tertiary dimethylaminoacetone ethylene ketal (VII; R=NMe_2), and dimethylaminoacetone was prepared by the hydrolysis of this ketal, or more simply by direct interaction of bromoacetone with aqueous dimethylamine.

Dimethylaminoacetone was quaternized with methyl iodide to observe the effect of the quaternary ammonium group on the infrared spectrum. The carbonyl frequency in (2-oxo-n-propyl)trimethylammonium iodide was located at 1728 cm^{-1} both in a Nujol mull or in chloroform solution, compared with that of acetone at 1710 cm^{-1} (as a liquid film or in chloroform solution), while dimethylaminoacetone absorbed at 1705 cm^{-1}.

These variations may be explained by the opposing effects on the carbonyl frequency of the neighbouring groups. The powerfully electron-attracting quaternary ammonium group reduces the tendency of the keto-group to enolize, and thus effectively shortens or strengthens the carbonyl double bond, causing an increase in the frequency of the infrared absorption similar to that observed with an α-halogen substituent.

Conversely, the dimethylamino group reduces the frequency of the infrared carbonyl absorption in a manner similar to that in *NN*-dialkylsubstituted amides. This may arise either from hydrogen-bonding in the enolic form, or possibly by the dimethylamino group acting as an electron source, increasing the contribution of the enolic form to the keto-enol tautomerism. Both effects will make the C=O double bond longer and weaker thereby reducing its absorption frequency. A similar shift to a lower frequency was observed in pinacolone, in which the strongly electron-repelling t-butyl group reduces the carbonyl frequency to 1705 cm^{-1}.

In agreement with the generalizations made by Bergmann and Pinchas (1952) and Lagrange and Mastagli (1955) regarding ethyleneglycol cyclic ketals, the several dioxolanes examined all exhibited characteristic infrared absorption in the 1000–1200 cm^{-1} region, with bands at about 1050, 1080, 1130, and 1200 cm^{-1}, of which the first was the most intense. Attempted reaction of dimethylaminoacetone ethylene ketal with 2-bromo-α-tetralone in acetone, benzene, or xylene solution to give the quaternary salt was unsuccessful, resulting

solely in dehydrobromination with the formation of α-naphthol and the tertiary amine hydrobromide.

In the case of the unprotected dimethylaminoacetone, it was possible to isolate the desired quaternary ammonium bromide, but again an appreciable amount of elimination occurred simultaneously.

Reactions of amines with 2-halo-α-tetralones and cyclohexanones to give substitution products are known (Hassner, Cromwell, and Davis 1957), but the corresponding elimination reaction often occurs also and the dehydrohalogenated compound may be the major or sole product when tertiary bases are used (Hassner, Cromwell, and Davis 1957). Thus 2-bromo-6-methyl-α-tetralone gave 6-methyl-1-naphthol when heated with diethylaniline (Fieser and Dunn 1936), 2-bromocyclohexanone and aniline gave cyclohexene (Koetz 1907), and 2-bromo-4,4-dimethyl-α-tetralone underwent both dehydrobromination and substitution by morpholine (Hassner and Cromwell 1958*b*).

In the non-polar solvents used, nucleophilic attack by the tertiary nitrogen may be expected to occur on the halogen-bearing carbon atom of the 2-bromo-α-tetralone, known from its ultraviolet and infrared absorption to possess the halogen in the axial conformation, as shown in (XXII).

The transition state formed on the approach of the tertiary nitrogen may then react in two ways: (i) by an S_N2 mechanism, to yield the quaternary ammonium salt, e.g. (XXI), or (ii) by the collapse of the transition state, to give a facile *trans*-diaxial elimination of hydrogen bromide resulting in the dienone (XI) which rearranges to 1-naphthol.

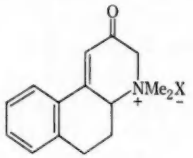
Steric hindrance due to the increased spatial requirements of the tertiary amines, compared with those of the corresponding secondary bases, particularly in the transition state formed from (XXII) and possibly also in the quaternary salt (XXI), will lead to an attack on the axial hydrogen atom in (XXII), and may be readily held to account for the high proportion of elimination observed in the reactions employing dimethylaminoacetone. This conclusion is supported by the facts that (i) the secondary base, methylaminoacetone ethylene ketal (VII; R=NHMe) reacted normally with 2-bromo-α-tetralone to give (46%) the substitution product (VIII); (ii) the tertiary base (VII; R=NMe_2) gave none of the substitution product, only elimination (43%) occurring; (iii) the spatially less-demanding dimethylaminoacetone gave both substitution (16·5%) and elimination (33%) products.

When the functional groups were reversed, and 2-dimethylamino-α-tetralone treated with bromoacetone, only dehydrobromination (29%) occurred. Steric considerations must again be operative in this instance, as the related secondary amine (XXIII) was reported to condense with bromoacetone by a substitution reaction in 40% yield (Kornfeld *et al.* 1956).

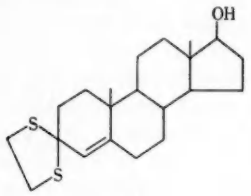
The quaternary diketone (XXI) showed infrared absorption at 3220 cm^{-1} (OH) suggesting that this material existed partially in the tricyclic hemiketal form (XXIV; R=H). Absorption bands were also present at 1675 cm^{-1} (aromatic C=O) and 1710 cm^{-1} (aliphatic C=O). The existence of the hemiketal (XXIV; R=H) was shown by its ready conversion to a methyl ether (XXIV;

R=Me) with methanolic hydrogen chloride. Similar hemiketals were reported by Kornfeld *et al.* (1956) in the indoloquinoline series.

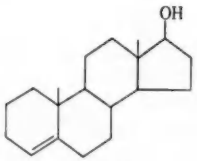
Cyclization of the quaternary diketone (XXI) was carried out by means of sodium methoxide in methanol or ethanol at −15 to −20 °C to give the tricyclic quaternary ketone (XXV; X=Br) which had infrared absorption identical with that of the methiodide obtained from (V), to which it was converted by means of sodium or potassium iodide in acetone. Mixed melting points established the identity of the two substances (XXV; X=I) and thus confirmed that the stereochemistry of the tricyclic ketone (V) and its quaternary salts (XXV) was the same by both methods of synthesis employed.



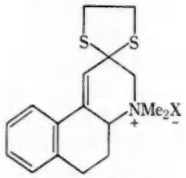
(XXV)



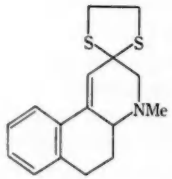
(XXVI)



(XXVII)



(XXVIII)



(XXIX)

The quaternary series, (XXI) and (XXV), were found to exhibit stability far superior to their tertiary analogues (IX) and (V). This was due not only to the greater ease of handling of crystalline solids rather than high-boiling liquids, but also to the electronic influence of the nitrogen substituent. Whereas the electron-attracting quaternary ammonium grouping $-\overset{+}{N}R_3$ stabilizes the neighbouring carbonyl system in the α-tetralone structure by resisting its enolization, the corresponding tertiary amine is able, by the use of its free electron pair, to weaken the carbonyl linkage and encourage enol formation, leading to ready aerial oxidation of the resulting dihydronaphthalene structure at neutral or alkaline pH values.

Attempts to form the dibenzyl mercaptal of the tricyclic quaternary ketone (XXV; X=Br) by reaction with benzyl thiol in presence of zinc chloride (Hauptmann 1947), dry hydrogen chloride or perchloric acid (d'Ouville, Myers, and Connor 1939; Hauptmann and Campos 1950), or boron trifluoride-etherate (Fieser 1954) were unsuccessful.

Using testosterone as a model for the αβ-unsaturated cyclic carbonyl system, reaction with ethanedithiol catalysed by boron trifluoride-etherate, gave a quantitative yield of the ethylene dithioketal (XXVI) which was satisfactorily desulphurized by means of Raney nickel to give the known Δ^4-androsten-17-ol (XXVII).

Using this method, the tricyclic quaternary ketone (XXV; X=Br) was converted to its ethylene dithioketal (XXVIII), characterized as the methopicrate.

Demethobromination of the quaternary dithioketal *in vacuo* was carried out by the method of Murphy and May (1954), May and Murphy (1955), and May and Fry (1957), and led to the amorphous tertiary dithioketal (XXIX). Evidence that the double bond in (XXIX) had not undergone isomerization into ring C to give a 3,4-dihydronaphthalene derivative was provided (i) by the fact that both the tertiary dithioketal and its hydrochloride showed an absorption maximum at 1630 cm^{-1}. The absence of a shift in the frequency of the C=C absorption on salt formation demonstrates that the double bond is in the βγ-position (Leonard and Gash 1954) whereas in αβ-unsaturated amines, salt formation is accompanied by a shift of *c.* 25 cm^{-1} due to the transformation $>C=C-N<$ to $>CH-C=\overset{+}{N}<$; (ii) by the presence in (XXIX) of an absorption band at 805 cm^{-1}, characteristic of a trisubstituted ethylene, but absent in tetrasubstituted olefins.

Desulphurization of (XXIX) with Raney nickel gave the amorphous 1-methyl-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (III; R=H), characterized as the picrate and (via the methiodide) as the methopicrate. Again both the tertiary base and its hydrobromide showed absorption maxima at 1605 cm^{-1}, demonstrating that the position of the βγ-double bond had not changed on desulphurization.

Further work in this field is in progress.

II. Experimental

Ultraviolet absorption spectra were determined in 95% ethanol on a Hilger "Uvispek" instrument. Infrared spectra were measured as capillary films or Nujol mulls unless otherwise indicated, on a Perkin-Elmer "Infracord" or Model 21 spectrophotometer, and melting points were taken on a Kofler block.

(*a*) *2-Bromo-α-tetralone.*—A stream of nitrogen was bubbled through a solution of α-tetralone (146 g; 1 mole) in carbon tetrachloride (500 ml) and a solution of bromine (160 g; 1 mole) in carbon tetrachloride was added dropwise. When the exothermic reaction had subsided, the mixture was warmed on the steam-bath for 1 hr and then distilled *in vacuo*, affording 2-bromo-α-tetralone (130·2 g, 59% yield) as a lachrymatory oil, b.p. 149–152 °C/2 mm, n_D^{22} 1·6135, which solidified to needles, m.p. 37–39 °C. Light absorption: $\nu_{max.}$ 1685 (aromatic C=O) and 642 cm^{-1} (axial C-Br).

(*b*) *Methylaminoacetone Ethylene Ketal.*—(i) A mixture of bromoacetone ethylene ketal (30 g: 0·165 mole) (b.p. 160 °C/710 mm, n_D^{23} 1·4752; Kuhn 1940) and liquid methylamine (80 g; 2·5 moles) was sealed in a 400 ml capacity autoclave and heated at 130 °C for 36 hr. The cooled mixture was stirred with ether and concentrated KOH soln., and the dried (sodium sulphate) ethereal extracts afforded on distillation methylaminoacetone ethylene ketal (2 g, 10% yield), b.p. 93 °C/70 mm, n_D^{23} 1·4340. Light absorption: $\nu_{max.}$ 1208, 1130, 1078, 1048 cm^{-1} (ketal).

(ii) A mixture of bromoacetone ethylene ketal (20 g; 0·11 mole) and ethanolic methylamine (100 ml, 30% v/v; 1 mole) was sealed in a 650 ml capacity autoclave and heated at 100 °C for

64 hr. Addition of 4 volumes of ether to the cooled solution precipitated methylamine hydrochloride (11·8 g, 95% yield). The filtrate was heated on the steam-bath to remove ethanol, and the residue taken up in ether, washed with conc. KOH, and dried over KOH. Distillation gave the ketal (9·06 g, 62% yield), b.p. 140–146 °C/735 mm, n_D^{21} 1·4321. Its hydrochloride had m.p. 166–167 °C; Kornfeld *et al.* (1956) give m.p. 165–167 °C.

(c) *Dimethylaminoacetone Ethylene Ketal.*—A mixture of bromoacetone ethylene ketal (30 g; 0·165 mole) and liquid dimethylamine (70 g; 100 ml; 1·55 moles) was sealed in a 650 ml capacity autoclave and heated at 170 °C for 26 hr. It was cooled, excess dimethylamine allowed to evaporate, and the contents diluted with ether and stirred with conc. KOH soln. The ether layer was combined with a subsequent ether-extract of the alkaline solution, dried over sodium sulphate, and distilled to give the ketal (13·0 g, 54% yield) as an oil, b.p. 83 °C/50 mm, n_D^{27} 1·4290. Light absorption: $\nu_{max.}$ 1208, 1165, 1130, 1095, and 1050 cm^{-1} (ketal).

The *hydrochloride* crystallized from ethanol–ether as needles of a hydrate, m.p. 161–162 °C (Found (after drying at 100 °C/2 mm): C, 45·4, 45·3; H, 8·8, 8·7; N, 7·6%. Calc. for $C_7H_{16}ClNO_2.0.25H_2O$: C, 45·3; H, 8·9; N, 7·5%).

The *hydrobromide* had m.p. 172–174 °C from ethanol–ether (Found: C, 36·8; H, 7·0; N, 6·0%. Calc. for $C_7H_{16}BrNO_2$: C, 37·2; H, 7·1; N, 6·2%).

(d) *Dimethylaminoacetone.*—(i) Dimethylaminoacetone ethylene ketal hydrochloride (1 g) was dissolved in 3N HCl (30 ml) and heated on a steam-bath for 8 hr. The solution was cooled, neutralized with dil. NaOH, and extracted with ether. The extract was dried over sodium sulphate and distilled, giving the base (0·42 g, 75% yield), b.p. 114 °C, n_D^{26} 1·4151.

(ii) Bromoacetone (70 g; 0·52 mole) was added dropwise with stirring and ice-cooling to a 26% w/v aqueous solution of dimethylamine (200 ml; 1·10 moles) during 1 hr. The dark solution was stirred overnight, then acidified with conc. HCl and non-basic material extracted with ether. (The ether solution yielded, on drying and evaporating, a trace of unchanged bromoacetone.) The acid solution was basified with sodium hydroxide and extracted with ether and chloroform. The combined extracts were dried over calcium chloride and distilled, affording the base (28·2 g, 54% yield), b.p. 65–75 °C/190 mm, n_D^{25} 1·4172. Light absorption: $\nu_{max.}$ 1705 cm^{-1} (C=O).

(iii) Chloroacetone (18·5 g; 0·20 mole) was added dropwise with stirring and cooling to a 10% excess of 33% w/v ethanolic dimethylamine (60 ml). The mixture was allowed to stand for 24 hr in a stoppered flask and evaporated to half its volume to remove dimethylamine. On cooling a precipitate of amine hydrochloride was deposited. The solution was basified and extracted with ether; distillation of the dried ether-extracts gave the amine (1·6 g, 8% yield), b.p. 124–125 °C, n_D^{31} 1·4080.

(e) *(2-Oxo-n-propyl)trimethylammonium Iodide.*—A mixture of dimethylaminoacetone (0·1 g), dry ethanol (10 ml), and methyl iodide in excess was refluxed for 3 hr. Cooling and addition of ether gave the *methiodide* (0·17 g, 68% yield), crystallizing from ethanol–ether as needles, m.p. 171–172 °C (Found: C, 29·5; H, 5·7%. Calc. for $C_6H_{14}INO$: C, 29·6; H, 5·8%). Light absorption: $\nu_{max.}$ 1728 cm^{-1} (C=O) (Nujol mull and $CHCl_3$ soln.).

(f) *2-[N-Methyl-N-(2'-oxo-n-propyl)]amino-α-tetralone 2'-Ethylene Ketal (VIII).*—A solution of methylaminoacetone ethylene ketal (9·0 g; 0·069 mole) and 2-bromo-α-tetralone (6·3 g; 0·028 mole) in benzene (50 ml) was refluxed on a steam-bath for 6·5 hr in a stream of nitrogen. The stoppered flask was kept at 0 °C overnight, the precipitated amino-ketal hydrobromide filtered, washed with benzene, and dried (6 g, 75% yield). The benzene filtrate on further heating deposited no more solid. It was cooled and washed with a little ice-water, then three times with cold 0·5N hydrochloric acid. The combined chilled acid extracts were immediately basified in a stream of nitrogen and the free base extracted into cold peroxide-free ether. The combined ether layers were dried at 0 °C over sodium sulphate and distilled, giving the tertiary amine (2·52 g, 46% yield), b.p. 125–135 °C/0·01 mm, n_D^{25} 1·5441 (Found: C, 69·5; H, 7·7; N, 4·8%. Calc. for $C_{16}H_{21}NO_3$: C, 69·8; H, 7·7; N, 5·1%). Light absorption: $\nu_{max.}$ 1687 (aromatic C=O), 1205, 1150, 1110, 1085, 1047 cm^{-1} (ketal).

Leemann and Fabbri (1959) report this compound but give no b.p. or refractive index.

The *picrate* crystallized from ethanol as yellow prisms, m.p. 145–147 °C (decomp.) (Found : C, 52·4; H, 4·7%; mol. wt. (method of Cunningham, Dawson, and Spring 1951), 500. Calc. for $C_{22}H_{24}N_4O_{10}$: C, 52·4; H, 4·8%; mol. wt., 504).

Treatment with alcoholic hydrogen bromide followed by dry ether gave the *hydrobromide* crystallizing from ethanol–ether as needles, m.p. 153–155 °C, of the hemihydrate (Found : C, 53·0; H, 6·3; N, 3·9%. Calc. for $C_{16}H_{22}BrNO_3.\frac{1}{2}H_2O$: C, 52·6; H, 6·3; N, 3·8%).

(*g*) *2-[N-Methyl-N-(2′-oxo-n-propyl)]amino-α-tetralone* (*IX*).—A solution of the preceding ethylene ketal (4·9 g; 0·018 mole) in ice-cold, nitrogen-saturated 3N HCl (90 ml) was sealed in a 100 ml flask and heated at 65±2 °C for 7 hr. The flask was chilled in ice, opened, and the contents basified in a stream of nitrogen with $NaHCO_3$. The free base was extracted four times as rapidly as possible with ice-cold peroxide-free ether, and the extracts dried over sodium sulphate at 0 °C. Distillation gave the amorphous *diketone* (2·81 g, 68% yield), b.p. 135–140 °C/10^{-5} mm, n_D^{19} 1·5845 (Found : C, 72·8; H, 7·3; N, 5·6%. Calc. for $C_{14}H_{17}NO_2$: C, 72·7; H, 7·4; N, 6·1%).

On standing, the liquid solidified to yellow crystals, m.p. 65–67 °C, which rapidly darkened on exposure to air. Light absorption : $\nu_{max.}$ 1710 (liq. film) or 1715 ($CHCl_3$) (aliphatic C=O) and 1690 cm^{-1} (aromatic C=O).

Alcoholic hydrogen bromide and ether gave the *hydrobromide*, crystallizing from ethanol–ether as a hemihydrate, m.p. 164–165 °C, even after drying *in vacuo* over P_2O_5 (Found : C, 52·3; H, 5·7%. Calc. for $C_{14}H_{18}BrNO_2.\frac{1}{2}H_2O$: C, 52·3; H, 6·0%).

The *methiodide* was deposited on refluxing an acetone solution of the amino-diketone under nitrogen with methyl iodide in excess for 2 hr. It crystallized from ethanol–ether as needles of a hemihydrate, m.p. 168–168·5 °C (Found : C, 47·2; H, 5·3; N, 4·2%. Calc. for $C_{15}H_{20}INO_2.\frac{1}{2}H_2O$: C, 47·1; H, 5·5; N, 3·7%).

(*h*) *1-Methyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinoline* (*V*).—Sodium methoxide (prepared from 0·60 g (0·026 mole) of sodium) was dissolved in dry methanol (7 ml) and the solution was added dropwise to a stirred solution of the preceding diketone (1·55 g; 0·0067 mole) in dry ethanol (25 ml) in an atmosphere of nitrogen at —20 °C. The temperature was kept at —10 °C for 15 min, then lowered to —25 °C while the sodium methoxide was decomposed by the addition of ice-water. The mixture was extracted three times with ether and chloroform, and the extracts washed with water to remove NaOH, dried over sodium sulphate at 0 °C, and evaporated. The residual amorphous cyclic ketone (0·525 g, 37% yield) had n_D^{22} 1·5950. Light absorption : $\nu_{max.}$ 1665 cm^{-1} (conjugated C=O). Leemann and Fabbri (1959) report $\nu_{max.}$ 1665 cm^{-1}.

The crude cyclization product (275 mg) was dissolved in absolute ethanol (4 ml) and treated with alcoholic hydrogen bromide until just acid. Addition of ether and crystallization from methanol–ether gave the *hydrobromide* as needles, m.p. 158–159 °C, of a hemihydrate even after drying at 60 °C/2 mm (Found : C, 55·7; H, 5·6; N, 4·7%. Calc. for $C_{14}H_{16}BrNO.\frac{1}{2}H_2O$: C, 55·5; H, 5·7; N, 4·6%). Light absorption : $\nu_{max.}$ 1650 cm^{-1} (conjugated C=O).

The crude cyclization product (70 mg) in absolute ethanol (5 ml) was refluxed with excess of methyl iodide for 1·5 hr. The mixture was chilled overnight and the solid filtered and recrystallized from ethanol, giving the *methiodide* as pale yellow needles, m.p. 198 °C (Found : C, 50·9; H, 5·3; N, 4·0%. Calc. for $C_{15}H_{18}INO$: C, 50·7; H, 5·1; N, 3·9%). Light absorption : $\nu_{max.}$ 1650 cm^{-1} (conjugated C=O).

(*i*) *2,3-Dihydro-1,3-dimethyl-3-hydroxy-5,6-(1′-2′-naphtho)-1,4-oxazine* (*XV*).—When the crude product obtained from an unsuccessful attempted cyclization of the diketone (IX) was kept at 0 °C overnight in the presence of dry HCl, crystallization of the product from methanol–ether gave the cyclic *hemiketal hydrochloride* as needles, m.p. 217–219 °C (Found : C, 63·3; H, 6·1; N, 5·8%. Calc. for $C_{14}H_{16}ClNO_2$: C, 63·3; H, 6·1; N, 5·3%). The compound was water soluble and gave an orange colour with ferric chloride solution. Light absorption : $\nu_{max.}$ 3300 (OH), 1600, 1230, 990, 930, 850, and 780 cm^{-1}.

(*j*) *3-Hydroxy-1-methyl-7,8-dihydro-5,6-benzquinolinium Hydroxide Betaine* (*XVIII*).—When the aqueous mother liquors from (*h*) above were made strongly alkaline with NaOH and extracted with chloroform, distillation of the extracts gave a solid residue (0·537 g) crystallizing from benzene–hexane as yellow needles, m.p. 129–130 °C, of the hydrated *betaine* (Found : C, 71·3;

H, 6·9%. Calc. for $C_{14}H_{13}NO.1·5H_2O$: C, 70·6 ; H, 6·8%). It was soluble in water and gave an orange colour with ferric chloride. Light absorption : $\nu_{max.}$ 3300 (OH), 1580, 1560, 1355, 1230, 1115, 1055, 890, 760, and 750 cm^{-1}.

(*k*) *Reaction of 2-Bromo-α-tetralone with Dimethylaminoacetone Ethylene Ketal.*—(i) Dimethylaminoacetone ethylene ketal (0·80 g ; 0·0055 mole) and 2-bromo-α-tetralone (1·25 g ; 0·0055 mole) in dry acetone (20 ml) were refluxed in a stream of nitrogen for 8 hr. On cooling, a solid (0·13 g) was deposited, filtered, and washed with ether. A further 0·11 g was obtained when the mother liquors were evaporated and the residue treated with ether. Recrystallization from ethanol–ether gave needles, m.p. 169–170 °C, of *dimethylaminoacetone ethylene ketal hydrobromide* (19% yield) (Found : C, 36·8 ; H, 7·0 ; N, 6·0%. Calc. for $C_7H_{16}BrNO_2$: C, 37·2 ; H, 7·1 ; N, 6·2%). Light absorption : $\nu_{max.}$ 1130, 1095, 1040 cm^{-1} (ketal) ; no absorption in the 1600–1700 cm^{-1} region.

(ii) Dimethylaminoacetone ethylene ketal (1·45 g ; 0·01 mole) and 2-bromo-α-tetralone (2·25 g ; 0·01 mole) in xylene (25 ml) were refluxed in nitrogen for 18 hr. On cooling, a solid was deposited, which was filtered, and washed with ether. It crystallized from ethanol–ether as needles (0·89 g, 40% yield), m.p. 169–170·5 °C, undepressed on admixture with the amino-ketal hydrobromide from (*i*) above.

The mother liquor from this amine hydrobromide was diluted with ether and extracted twice with dil. NaOH solution. The alkaline extract was acidified with HCl and the acid extracted with chloroform. Removal of solvent gave a crystalline residue (0·62 g, 43% yield), m.p. 85–91 °C, identified as α-naphthol by giving a characteristic blue colour when warmed in sodium hydroxide solution with chloroform and copper bronze, and by the formation of the picrate (orange needles from ethanol), m.p. and mixed m.p. 189–189·5 °C.

(*l*) *NN-Dimethyl-N-(α-oxo-2-tetralyl)-N-(2′-oxo-n-propyl)ammonium Bromide (XXI).*—(i) A solution of dimethylaminoacetone (7·0 g ; 0·069 mole) and 2-bromo-α-tetralone (15·5 g ; 0·069 mole) in dry acetone (70 ml) was refluxed in nitrogen for 17 hr. A solid began to precipitate during this period. After cooling, the solid (2·5 g) (m.p. 194–195 °C) was filtered and washed with acetone. The acetone mother liquors on standing for several days at room temperature deposited a further 1·18 g (m.p. 194 °C). Crystallization from ethanol–ether gave the *quaternary bromide* (16·3% yield), m.p. 197 °C (Found : C, 55·1, 55·0 ; H, 6·3, 6·0 ; N, 4·1%. Calc. for $C_{15}H_{20}BrNO_2$: C, 55·2 ; H, 6·2 ; N, 4·3%. Calc. for $C_{15}H_{18}BrNO_2$: C, 55·9 ; H, 5·6%). Light absorption : $\nu_{max.}$ 3220 (OH), 1710 (aliphatic C=O), 1675 cm^{-1} (conjugated C=O).

(ii) After an identical experiment using 2·85 g of dimethylaminoacetone, the mother liquor from the quaternary bromide was concentrated and the residue chilled in an attempt to increase the yield. The solid, m.p. 81–85 °C, produced (1·7 g, 33% yield) was filtered and washed with acetone. It was water-soluble, contained ionic halide, and was *dimethylaminoacetone hydrobromide* (Found : C, 32·6 ; H, 6·8%. Calc. for $C_5H_{12}BrNO$: C, 33·0 ; H, 6·7%).

(*m*) *Reaction of 2-Dimethylamino-α-tetralone with Bromoacetone.*—2-Dimethylamino-α-tetralone (0·80 g ; 0·0042 mole) and bromoacetone (0·58 g ; 0·0042 mole) in dry acetone (50 ml) were refluxed in nitrogen for 3 hr. There was no precipitate on cooling. The solution was evaporated to dryness, and the residue recrystallized from acetone–ether, giving *2-dimethylamino-α-tetralone hydrobromide* (0·35 g, 29% yield), m.p. 81–84 °C as a hydrate (Found : C, 49·6 ; H, 6·3 ; N, 4·3 ; Br, 27·9%, equiv. wt., 287. Calc. for $C_{12}H_{20}BrNO.H_2O$: C, 50·0 ; H, 6·3 ; N, 4·8 ; Br, 27·7% ; equiv. wt., 288).

(*n*) *2,3-Dihydro-1,3-dimethyl-3-methoxy-5,6-(1′,2′-naphtho)-1,4-oxazine Methobromide (XXIV ; R=Me).*—A solution of the quaternary ammonium compound, m.p. 194 °C (100 mg), obtained in (*l*) above in dry methanol (6 ml) was saturated with dry hydrogen chloride, and kept at room temperature for 19 hr. Removal of solvent and crystallization of the residue from methanol–ether gave needles (61 mg, 59% yield), m.p. 154–155 °C, of the hydrated *methyl ether*, not dehydrated on drying *in vacuo* over P_2O_5 (Found : C, 54·1, 54·4 ; H, 6·7, 7·0%. Calc. for $C_{16}H_{22}BrNO_2.H_2O$: C, 53·6 ; H, 6·8%. Calc. for $C_{16}H_{22}BrNO_2.0·75H_2O$: C, 54·3 ; H, 6·7%). The analysis was unchanged after drying at 80 °C/3 mm (Found : C, 54·3 ; H, 6·8%). After recrystallization from isopropanol–ether, and drying at 75 °C/3 mm for 3 hr it had m.p. 160–161 °C (Found : C, 56·5 ; H, 6·7 ; N, 3·9%. Calc. for $C_{16}H_{22}BrNO_2$: C, 56·5 ; H, 6·5 ; N, 4·1%).

(*o*) *Cyclization of Quaternary Diketone.*—The quaternary diketone (2·28 g; 0·007 mole) obtained in (*k*) above was dissolved in dry methanol (200 ml) and the solution stirred in a current of nitrogen at —15 °C. A solution of sodium methoxide (prepared from 0·48 g (0·021 mole) of Na) in methanol (50 ml) was added to the diketone solution and the mixture stirred at —15 °C for 0·5 hr. Sodium methoxide in excess was neutralized by addition of ethanolic hydrogen bromide to pH 6 and the solution evaporated to dryness at reduced pressure. The dry residue was extracted three times with boiling anhydrous ethanol. The ethanol extracts when diluted with ether deposited the tricyclic *quaternary ketone* (XXV; X=Br) (1·27 g, 59% yield), m.p. 198–200 °C, from methanol (Found: C, 58·0; H, 6·1; N, 4·5; Br, 29·4%. Calc. for $C_{15}H_{16}BrNO$: C, 58·4; H, 5·9; N, 4·6; Br, 29·9%). Its infrared spectrum, $\nu_{max.}$ 1650 cm^{-1} (conjugated C=O), was practically identical with that of the methiodide, m.p. 198 °C, obtained in (*h*) above.

Extraction of the ethanol-insoluble residue with boiling anhydrous methanol afforded a further crop (0·3 g) of the product, m.p. and mixed m.p. 198 °C, with identical infrared absorption, a total of 1·57 g (73% yield). Recrystallization of this material from methanol–ether gave needles of a hemihydrate, m.p. 189–190 °C (Found: C, 57·0; H, 6·2%. Calc. for $C_{15}H_{18}BrNO.\frac{1}{2}H_2O$: C, 56·8; H, 6·0%).

(*p*) *Conversion of the Tricyclic Ketone Methobromide to the Methiodide.*—(i) A dry acetone extract (50 ml) of the crude cyclization product obtained in (*o*) above was refluxed for 1 hr with KI (100 mg). A white solid was deposited almost immediately and shown to be KBr (m.p. above 250 °C). The mother liquor was evaporated to dryness, and the residue recrystallized from ethanol to give needles, m.p. 198–199 °C, undepressed on admixture with the methiodide (m.p. 198 °C) of 1-methyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinoline obtained in (*h*) above.

(ii) In another experiment, the pure cyclization product obtained in (*o*) above (83 mg; 0·00027 mole) was dissolved in dry acetone and refluxed with NaI (38 mg; 0·00025 mole) for 1 hr. It was evaporated to 50 ml, cooled, and the trace of solid which separated was filtered. The filtrate was evaporated to dryness, leaving a residue (85 mg) which crystallized from ethanol–ether as needles, m.p. 196–197 °C, identical (mixed m.p. and infrared spectrum) (absorption bands at 2290, 1650, 1580, 1300, 1230, 1180, 760, and 720 cm^{-1}) with the methiodide (m.p. 198 °C) obtained in the above.

(*q*) *1,1-Dimethyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinolinium Bromide Ethylene Dithioketal (XXVIII; X=Br).*—(i) The tricyclic quaternary ketone (XXV; X=Br) (0·42 g; 0·0013 mole) was stirred with ethanedithiol (0·25 ml; 0·0026 mole) and boron trifluoride etherate (0·15 ml) The mixture, which became slightly warm, was stoppered and set aside for 2½ days at room temperature. It was diluted with ether and the grey solid filtered and washed with ether to give 496 mg (94% yield) of crude product (m.p. 218–220 °C). Recrystallization from ethanol gave white needles (0·322 g, 62% yield), m.p. 230–231 °C, of the *dithioketal* as a hemihydrate (Found: C, 52·1; H, 5·6; N, 3·6; S, 16·6%. Calc. for $C_{17}H_{22}BrNS_2.\frac{1}{2}H_2O$: C, 51·9; H, 5·9; N, 3·6; S, 16·3%). Light absorption: $\nu_{max.}$ 1620 cm^{-1}.

(ii) In an identical experiment, recrystallization of the product from methanol gave needles, m.p. 185 °C, of the monohydrate (Found: C, 50·4; H, 5·8; N, 3·3; Br, 20·1%. Calc. for $C_{17}H_{22}BrNS_2.H_2O$: C, 50·7; H, 6·0; N, 3·5; Br, 19·9%), showing infrared absorption identical with that of the compound obtained in (*q*) (i).

(iii) Treatment of an aqueous solution of the above methobromide with lithium picrate gave the *methopicrate* as needles from aqueous ethanol, m.p. 218–222 °C (Found: C, 51·4; H, 4·8%. Calc. for $C_{23}H_{24}N_4S_2O_7.\frac{1}{2}H_2O$: C, 51·0; H, 4·7%).

(*r*) *1-Methyl-3-oxo-1,2,3,7,8,9-hexahydro-5,6-benzquinoline Ethylene Dithioketal (XXIX).*—The quaternary dithioketal obtained above in (*q*) (215 mg; 0·00056 mole) was heated at 215 °C (bath temp.) *in vacuo* (0·7 mm) for 10 min. The yellowish amorphous residue of the tertiary amine (177 mg) showed light absorption ($\nu_{max.}$) at 1630 cm^{-1}.

The hydrochloride, prepared from an ethereal solution of the amine with dry ethereal hydrogen chloride, similarly had light absorption ($\nu_{max.}$) at 1630 cm^{-1}, demonstrating that the double bond was in the $\beta\gamma$-position.

The base was used without purification for the next step.

(*s*) *1-Methyl-1,2,3,7,8,9-hexahydro-5,6-benzquinoline (III; R=H).*—The tertiary dithioketal obtained above in (*r*) (177 mg) was refluxed for 16 hr with Raney nickel (1 g) in ethanol (25 ml).

The catalyst was removed, washed with hot ethanol and ether, and the filtrate and washings evaporated, affording the amorphous hexahydrobenzquinoline (92 mg, 76% yield). Light absorption : $\nu_{max.}$ 1605 cm^{-1}. The hydrobromide, prepared using dry ethereal hydrogen bromide, similarly showed light absorption ($\nu_{max.}$) at 1605 cm^{-1}, demonstrating that the double bond was in the $\beta\gamma$-position.

The *picrate* formed needles from aqueous methanol, m.p. 176–177 °C (Found : C, 55·9 ; H, 4·5% ; mol. wt. (method of Cunningham, Dawson, and Spring 1951), 449. Calc. for $C_{30}H_{26}N_4O_7$: C, 56·1 ; H, 4·7% ; mol. wt., 428). Light absorption : $\nu_{max.}$ ($CHCl_3$ solution) 1605 cm^{-1}. Treatment of the amorphous tertiary base with methyl iodide followed by double decomposition with aqueous lithium picrate gave the *methopicrate*, crystallizing from water as needles, m.p. 208–210 °C (Found : mol. wt. (method of Cunningham, Dawson, and Spring 1951), 460. Calc for $C_{21}H_{22}N_4O_7$: mol. wt., 442).

(*t*) *Testosterone Ethylene Dithioketal.*—Testosterone (203 mg) was stirred with ethanedithiol (0·15 ml) and boron trifluoride etherate (0·1 ml). The solution warmed spontaneously and soon solidified. After standing for 1 hr it was diluted with methanol (5 ml), filtered, and dried. The *dithioketal* (260 mg, 100% yield) crystallized from ethanol as needles of a hemihydrate which sintered at 100–105 °C and melted at 163–165 °C (Found : C, 67·9 ; H, 9·0%. Calc. for $C_{21}H_{32}OS_2.\frac{1}{2}H_2O$: C, 67·5 ; H, 8·9%), obtained anhydrous after drying at 100 °C/2 mm for 16 hr (Found : C, 68·9 ; H, 8·8%. Calc. for $C_{21}H_{32}OS_2$: C, 69·2 ; H, 8·8%). Light absorption : $\nu_{max.}$ 3350 (OH), 2350, 1650, 1270, 1225, 1070, 1050, 850, 835, 725, and 670 cm^{-1}.

(*u*) *Δ-4-Androsten-17-ol.*—Testosterone ethylene dithioketal (70 mg) in ethanol (20 ml) was refluxed for 2 hr with Raney nickel (2 g). The solution was filtered and the catalyst washed twice with boiling ethanol. The combined filtrate and washings were evaporated, leaving a white solid (70 mg), crystallizing from methanol or ethanol as needles, m.p. 147 °C (lit. m.p. 146–149 °C). With tetranitromethane the compound gave a positive test for unsaturation, and Lassaigné's test for sulphur was negative (Found (after drying at 60 °C/2 mm overnight) : C, 80·1 ; H, 10·7%. Calc. for $C_{19}H_{30}O.\frac{1}{2}H_2O$: C, 80·5 ; H, 11·0%). Light absorption : $\nu_{max.}$ 3350 (OH), 2350, 1115, 1070, 1050, 1000, 815, and 670 cm^{-1}.

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THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *FLINDERSIA* SPECIES

XIV. THE CONSTITUENTS OF FLINDERSIA PUBESCENS BAIL. AND F. SCHOTTIANA F. MUELL.

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Summary

The alkaloids and other constituents of *Flindersia pubescens* Bail. and *F. schottiana* F. Muell. have been isolated. From *F. schottiana* has been obtained osthol hydrochloride and chemical work on its genesis is described. Several substances related to osthol are reported for the first time.

I. Introduction

Flindersia pubescens Bail. occurs typically on the Atherton Tableland and adjoining areas of north Queensland. It is commonly known as silver ash and the timber is used as a building material, for plywood, and for "blonde" furniture. *F. schottiana* F. Muell. is a valuable commercial timber, popularly known as southern silver ash or bumpy ash, and is found in the coastal scrubs from the Hastings River, N.S.W., northward into south-eastern Queensland. Mr. L. S. Smith of the Queensland Botanic Museum and Herbarium, Brisbane, has written as follows on the classification of the two species:

"*Flindersia schottiana* and *F. pubescens* are generally accepted as two very closely related species differing little except in length and density of the indumentum, although *F. pubescens* does tend to have slightly larger leaves and fruit . . . It is possible that when more complete botanical material is available throughout the whole range of distribution, both of the above may prove to be variants within a single polymorphic species (*F. schottiana*)."

II. Discussion

Contrary to this botanical opinion, the two species have proved to be somewhat different chemically, as shown in Tables 1 and 2. The substances were isolated by systematic extraction in the usual way.

The alkaloid, $C_{16}H_{15}O_2N$, m.p. 138 °C, appears from its spectral properties to be a furoquinoline type; but in view of the difficulty of separation of these alkaloids and the small amount available, its homogeneity must still be considered suspect. The other bases were substances of known structure, apart from maculosidine, the isolation of which at this stage allowed its structure to be resolved (Prager, Ritchie, and Taylor 1960). Details of a paper chromatographic system for furoquinolines are given in Section III.

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The isolation of osthol hydrochloride (Ia), which was readily identified by elemental analysis and direct comparison, was most surprising. The prior treatment of the extract with 5% hydrochloric acid to separate alkaloids strongly suggested that the compound was an artefact formed during this process, osthol or osthol hydrate (Ib) being possible precursors. On mechanistic grounds osthol was not seriously considered, and indeed it was recovered unchanged when treated under conditions similar to those employed in the work-up of the extraction.

TABLE 1

THE CONSTITUENTS OF F. PUBESCENS

Substance	Bark (%)	Leaves (%)	Wood (%)
Sitosterol	0·001	0·006	—
Osthol	0·34	—	—
Sesamin	0·008	—	—
Dictamnine	Trace	—	—
Flindersiamine	Trace	—	—
Kokusaginine	—	0·004	Trace
Maculosidine	—	0·005	—
Skimmianine	Trace	—	—
$C_{16}H_{16}O_2N$, m.p. 138 °C ..	Trace	—	Trace

TABLE 2

THE CONSTITUENTS OF F. SCHOTTIANA

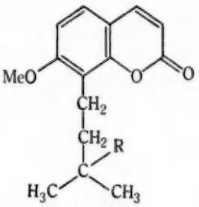
Substance	Bark (%)	Leaves (%)	Wood (%)
Sitosterol	0·02	0·0003	0·004
Osthol	0·4	0·0004	0·0001
Osthol hydrochloride	0·06	—	—
Kokusaginine	0·0004	0·005	—
Maculine	0·009	0·008	0·002

Osthol hydrate (Ib) seemed a more likely precursor, and so its synthesis was examined. Treatment of osthol hydrochloride with silver acetate gave a non-crystalline acetate, but attempted hydrolysis of this yielded mainly osthol by an elimination process. However, it was eventually found that hydrolysis of the corresponding trifluoroacetate under mild conditions yielded osthol hydrate, together with much osthol which was readily separated by chromatography.

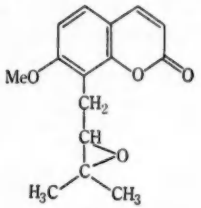
Osthol hydrate crystallized as a monohydrate, the anhydrous material being a gum for which a satisfactory analysis could not be obtained. However, its structure was confirmed by its conversion to osthol hydrochloride on treatment with dry hydrogen chloride. On the other hand, osthol hydrate was recovered completely unchanged after being shaken in ethereal solution with 5% hydro-

chloric acid for 15 hr. It therefore seems unlikely that the hydrate was the precursor of the hydrochloride in *F. schottiana*.

In a further attempt to elucidate the origin of osthol hydrochloride, two more samples of *F. schottiana* bark were extracted. In one, the constituents were separated by means of chromatography, without recourse to acid treatment, while in the other 5% hydrochloric acid was again used for the initial separation of alkaloids. In neither case was the presence of osthol hydrochloride or osthol hydrate detected. Although osthol was isolated from both specimens, the question whether osthol hydrochloride is an artefact therefore remains open.



(Ia; R=Cl)
(Ib; R=OH)



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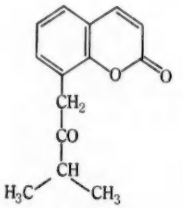
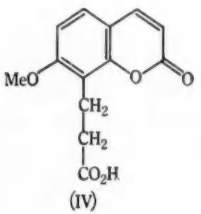


(III)



(IV)

Other routes to osthol hydrate tentatively explored involved osthol epoxide (II) and homo-ostholic acid (IV), preparations of which are reported here for the first time. (+)-Osthol epoxide occurs in orange peel oil as aurapten (Böhme and Pietsch 1939). The (±)-epoxide was prepared by selective epoxidation of osthol. However, while it was expected that reductive fission of the oxirane would give the tertiary alcohol (Newman 1956), catalytic hydrogenation and chemical methods were either ineffective or, under more forcing conditions, produced attack on the lactone ring; on acid treatment the known ketone (III) was formed. Homo-ostholic acid was prepared by Arndt-Eistert homologation of ostholic acid. The reaction of the derived acid chloride with dimethylcadmium or methylmagnesium iodide gave unsatisfactory results, and the work was discontinued when the more direct route to the hydrate was developed.

III. Experimental

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. Ultraviolet spectra were measured in purified ethanol on a Hilger Uvispek. Infrared spectra of substances as mulls in paraffin were recorded on a Perkin-Elmer Infracord 137. Analyses were performed by Miss B. Stevenson of these Laboratories, and by the C.S.I.R.O. and the University of Melbourne Microanalytical Laboratories, Melbourne. The general procedure for extraction and isolation that was followed has been outlined in Part XII (Ritchie, Taylor, and Willcocks 1960). Substances isolated were identified by direct comparison (mixed m.p.'s and infrared spectra) with authentic specimens.

(*a*) *Extraction of the Bark of* F. pubescens.—The dried milled bark (26 kg) (S.N.5937, collected at Danbulla, north Queensland) was exhausted by percolation at room temperature in turn with light petroleum, ether, acetone, and methanol. The concentrated light petroleum extract deposited osthol (86 g) on standing. The alkaloids isolated with 5% HCl from the light petroleum and ether extracts (2 g and 0·5 g respectively) were combined, as were those from the acetone and methanol (0·5 g each). The alkaloids were separated by repeated chromatography on alumina, giving pure specimens of dictamnine (10 mg), flindersiamine (5 mg), and skimmianine (15 mg); a fraction of constant m.p. 186 °C proved to be bourjotine since, on careful chromatography on a large amount of alumina, flindersiamine, and skimmianine could be partly separated. The mother liquors of the bourjotine crystallization on further chromatography yielded a new *base* (0·3 g), m.p. 138 °C, after repeated crystallizations from aqueous methanol and then cyclohexane (Found: C, 76·4; H, 6·0; N, 5·9; O, 12·2%. Calc. for $C_{16}H_{15}O_2N$: C, 76·0; H, 5·9; N, 5·5; O, 12·7%). Light absorption: $\lambda_{max.}$ 215, 248, 317, and 337 mμ, log ε 4·6, 4·64, 4·24, and 4·29 respectively.

Paper chromatography of mother liquors did not indicate the presence of alkaloids other than those isolated. The bases were run on paper buffered to pH 2 with citric acid, with cyclohexane–benzene (40 : 60) as the mobile phase. Detection was by means of ultraviolet light. Typical R_F values (circular chromatography) are as follows:

Flindersiamine	0·76	Skimmianine	0·68	Maculosidine	0·62
Maculine	0·89	Dictamnine	0·92	Kokusaginine	0·63
Maculosine	0·00	$C_{16}H_{15}O_2N$, m.p. 138 °C			0·43

Negligible acidic and phenolic fractions were obtained.

Hot saponification of the neutral fraction from the light petroleum extract gave a neutral fraction from which (+)-sesamin (2 g) was obtained by crystallization from methanol, m.p. and mixed m.p. 125 °C (Found: C, 67·5; H, 5·3%; OCH_3, nil. Calc. for $C_{20}H_{18}O_6$: C, 67·7; H, 5·1%), $[\alpha]_D$ +48·2° (*c*, 0·98) (lit. +68°, indicating possibly slight racemization). Chromatography of the mother liquors on alumina yielded sitosterol (0·2 g). Likewise, the ether extract gave sesamin (0·1 g); the acetone extract, osthol (0·5 g); the methanol extract, osthol (0·2 g), sesamin (0·1 g), and sitosterol (0·1 g).

(*b*) *Extraction of the Leaves of* F. pubescens.—The leaves (15·1 kg) were extracted successively with light petroleum, ether, acetone, and methanol. The ether extract yielded sitosterol (1 g) and maculosidine (2·5 g) and kokusaginine (0·25 g). No crystalline material was isolated from the light petroleum, acetone, or methanol extracts.

(*c*) *Extraction of the Wood of* F. pubescens.—The wood (12 kg) was treated as in (*a*) above. The combined alkaloid fraction gave kokusaginine (0·2 g) and the $C_{16}H_{15}O_2N$ alkaloid isolated from the bark (0·1 g).

(*d*) *The Extraction of the Bark of* F. schottiana.—The bark (11·5 kg) (S.N.5887 collected at Whian Whian, N.S.W.) was treated as in (*a*) above.

The concentrated light petroleum extract deposited osthol (49 g) on standing; the combined light petroleum and ether alkaloid fraction (2·7 g) gave maculine (0·56 g) on chromatography, while the acetone and methanol extracts gave maculine (0·28 g) and kokusaginine (0·042 g).

The light petroleum neutral fraction (40 g) was chromatographed on alumina (800 g). Elution with benzene gave osthol hydrochloride (8·9 g) and ether-eluted sitosterol (2·0 g).

The ether neutral fraction (12 g) yielded osthol (0·5 g) and sitosterol (0·12 g), the acetone neutral fraction (7 g), osthol (0·052 g) and sitosterol (0·03 g), and the methanol extract (7·1 g), osthol (0·03 g).

(*e*) *Extraction of the Leaves of* F. schottiana.—The leaves (14·2 kg) (S.N.5887 and S.N.6019 collected at Whian Whian) were treated as in (*a*) above. The light petroleum extract gave osthol (0·32 g), sitosterol (0·04 g), maculine (0·55 g), and kokusaginine (0·07 g); the ether extract, maculine (0·43 g) and kokusaginine (0·72 g), and the methanol extract, maculine (0·03 g).

(*f*) *Extraction of the Wood of* F. schottiana.—The wood (19 kg) (S.N.5887) was treated as in (*a*) above, the acetone extraction being omitted. The light petroleum extract gave sitosterol (0·6 g) and maculine (0·2 g), the ether extract, osthol (0·03 g) and maculine (0·03 g), and the methanol extract, sitosterol (0·04 g) and maculine (0·04 g).

(*g*) *Osthol Hydrochloride.*—This crystallized as colourless blades from light petroleum, m.p. 98–99 °C (Found: C, 64·3; H, 6·2; O, 17·3; Cl, 12·4; OMe, 10·9%. Calc. for $C_{15}H_{17}O_3Cl$: C, 64·2; H, 6·2; O, 17·1; Cl, 12·5; 1×OMe, 11·0%). It was readily prepared by treating osthol in chloroform with dry hydrogen chloride.

(*h*) *Osthol Hydrate.*—Osthol hydrochloride (0·6 g) in benzene (15 ml) was shaken in the dark with silver trifluoroacetate (0·6 g) for 4 hr. Methyl iodide (1 g) was added to remove the excess silver trifluoroacetate and the mixture filtered. The filtrate was evaporated to dryness to give a clear gum with strong bands in the infrared at 1780 (trifluoroacetate) and 1730 cm^{-1} (coumarin lactone). Hydrolysis was effected by treating the ester in methanol (30 ml) with $NaHCO_3$ (200 mg) in water (2 ml). After 2 days at room temperature the solution was evaporated to dryness *in vacuo* and the residue worked up in the usual way to give a product which was chromatographed on silica gel (40 g). Elution with benzene gave osthol (0·45 g) and benzene–ether (70 : 30) gave osthol hydrate (60 mg). The recovered osthol was recycled to give, eventually, 120 mg of material, needles from ether, m.p. 83–86 °C (Found: C, 64·1; H, 7·1%. Calc. for $C_{15}H_{18}O_4.1H_2O$: C, 64·2; H, 7·1%). On drying *in vacuo* even at room temperature, water of crystallization was lost to give a clear gum which could not be obtained completely anhydrous (Found: C, 67·6; H, 6·8%. Calc. for $C_{15}H_{18}O_4$: C, 68·6; H, 6·9%). On treatment with dry HCl in chloroform osthol hydrochloride was obtained quantitatively.

A solution of osthol hydrate (65 mg) in ether (25 ml) was shaken with 5% HCl soln. (25 ml) for 15 hr. The product obtained after work up in the usual way gave a negative Beilstein test; it was chromatographed on alumina to give negligible material on elution with benzene, but on elution with benzene–ether (50 : 50), osthol hydrate (55 mg). Similarly, osthol was recovered unchanged after treatment with 5% HCl soln. for 3 days.

(*i*) *Osthol Epoxide.*—Osthol (2 g; 0·00082 mole) in chloroform (30 ml) was kept at 0 °C for 7 days with perbenzoic acid (0·009 mole). Excess peracid was then removed with KI–$Na_2S_2O_3$, and the solution worked up in the usual way to give the epoxide (1·9 g), needles from methanol, m.p. 105–106 °C. For analysis it was sublimed at 195 °C/0·01 mm, giving needles, m.p. 117–118 °C (Found: C, 69·1; H, 6·1%. Calc. for $C_{15}H_{16}O_4$: C, 69·2; H, 6·2%).

Osthol epoxide was inert to mild hydrogenation conditions, such as palladized charcoal, but stronger conditions such as hydrogen in presence of platinic oxide/perchloric acid gave ill-defined products whose spectral properties suggested hydrogenation of the lactone ring. Sodium borohydride at room temperature in refluxing methanol had no effect; lithium aluminium hydride attacked the lactone ring.

(*j*) *Isomerization of Osthol Epoxide.*—The epoxide (0·1 g) was refluxed for 4 hr with 20% H_2SO_4 (20 ml). Extraction with chloroform gave a crude product which was chromatographed on alumina; elution with benzene–ether (99 : 1) gave the ketone (III), needles from water, m.p. 63 °C (lit. m.p. 66 °C).

(*k*) *Homo-ostholic Acid.*—Ostholic acid (1 g) was refluxed with thionyl chloride (10 ml) for 1·5 hr, when excess was distilled off *in vacuo*. The residue was taken up in benzene (15 ml) and the solution concentrated to one-half volume; addition of dry ether gave ostholyl chloride, needles from benzene–ether, m.p. 222–223 °C. An ethereal suspension of the chloride was added slowly with stirring to ethereal diazomethane (80 ml) prepared from *N*-nitrosomethylurea (10 g).

After stirring for 3 hr the precipitated diazoketone was collected and decomposed as follows: A solution of silver benzoate (0·1 g) in dry triethylamine (10 ml) was added dropwise to a solution of the diazoketone in methanol (25 ml) over 0·5 hr at 55 °C. After stirring for a further 1·5 hr and heating to 100 °C for 3 min the solution was charcoaled and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue, in benzene, chromatographed on alumina. Elution with benzene–ether (95 : 5) yielded methyl homo-ostholate (0·7 g), needles from benzene–ether, m.p. 135–136 °C (Found: C, 60·4; H, 5·1%. Calc. for $C_{14}H_{14}O_5$: C, 60·7; H, 4·9%). Hydrolysis of the ester with 3N HCl in dioxan yielded homo-ostholic acid, purified by sublimation at 220 °C/0·4 mm followed by crystallization from methanol-ethyl acetate as plates, m.p. 238–240 °C (Found: C, 62·8; H, 5·0%. Calc. for $C_{13}H_{12}O_5$: C, 62·9; H, 4·9%).

(*l*) *Further Extractions of Bark of* F. schottiana.—(i) The bark (9·1 kg) (S.N.6035) was extracted with light petroleum. The concentrated extract was chromatographed in benzene on alumina (4 kg). The material eluted with benzene (10 l.) was rechromatographed in light petroleum on alumina; elution with benzene gave a mixture of coumarins and alkaloids which were separated by chromatography on silica gel, elution with benzene giving osthol (25 g, 0·3%). Thorough examination of all mother liquors did not reveal the presence of any osthol hydrochloride or osthol hydrate.

(ii) The bark (8·6 kg) (S.N.6019) was extracted as in (*a*) above to give osthol (24 g, 0·27%).

IV. Acknowledgments

The authors are indebted to Mr. W. T. Jones, C.S.I.R.O., Brisbane, for supplying the plant material, to Mr. L. S. Smith of the Queensland Botanic Museum and Herbarium, Brisbane, for botanical information, to Professor H. G. H. Erdtman, Stockholm, for a specimen of sesamin, and to the C.S.I.R.O. for the award of scholarships to A.F.H. and R.H.P.

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THE CHEMICAL CONSTITUENTS OF *HIMANTANDRA* (*GALBULIMIMA*) SPECIES

IV. THE STRUCTURES OF HIMBACINE, HIMBELINE, HIMANDRAVINE, AND HIMGRAVINE

By J. T. Pinhey,* E. Ritchie,* and W. C. Taylor*

[*Manuscript received July* 18, 1960]

Summary

By an extensive series of degradations involving dehydrogenation, exhaustive methylation, and oxidation, the structure of himbacine is established. It is the first member of a new group of alkaloids. By simple transformations, himbeline, himandravine, and himgravine are shown to be closely related to himbacine and their structures also established.

I. Introduction

When the previous parts of this series (Hughes and Ritchie 1954; Brown *et al.* 1956; Moyle and Ritchie 1958) were published the authors were under the impression that the name *Himantandra* was to be preferred to *Galbulimima* as the name of the genus, but it now appears that the reverse is the case. The situation with respect to nomenclature has been summarized by Mr. L. S. Smith of the Queensland Botanic Museum and Herbarium, Brisbane, as follows: "There has been some disagreement as to the naming of the genus and also as to the number of species present in New Guinea. A. C. Smith (1942) considers that the name *Himantandra* is more consistent and he recognizes only one species, *H. belgraveana* (F. Muell.) Diels in New Guinea. However there can be no doubt that according to the existing International Code of Botanic Nomenclature (1956), *Galbulimima* F. M. Bail. is the correct name for the genus of rain-forest trees sometimes called *Himantandra* F. Muell. ex Diels and Himantandraceae is the correct name for the family.

"Four species have been described, one from Queensland and three from New Guinea. Relying only on descriptions of two of these species, A. C. Smith has suggested that only two species may really be involved, one in Queensland and one (or possibly both) in New Guinea. Names of the species and their authors follow.

Queensland: *Galbulimima baccata* F. M. Bail. [=*Himantandra baccata* (F. M. Bail.) Diels].

New Guinea: *Galbulimima belgraveana* (F. Muell.) Sprague [=*Himantandra belgraveana* (F. Muell.) Diels], the common species.

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Galbulimima nitida (Bak. f. and Norman) Sprague [=*Himantandra nitida* Bak. f. and Norman]. On the basis of description only and without seeing specimens this has been reduced by A. C. Smith to a synonym of the preceding species.

Galbulimima parvifolia (Bak. f. and Norman) Sprague [=*Himantandra parvifolia* Bak. f. and Norman].

From the description only, again A. C. Smith has stated that the leaves suggest *G. baccata*, though if not distinct it might be expected to be a depauperate form of *G. belgraveana* on the grounds of locality."

In any future publications in this series, the genus will be referred to as *Galbulimima* and the term *Himantandra* will not be used.

II. Functional Groups of Himbacine

Himbacine, $C_{22}H_{35}O_2N$, was optically active and was quite a strong base, the pK_a being 9·3 in 50% ethanol. Analyses showed it to contain no methoxyl group, one *N*-methyl group, and no active hydrogen; terminal methyl group estimations gave values ranging from 0·91 to 1·39, indicating the presence of two such groups.

The alkaloid did not react with acylating reagents and its i.r. spectrum confirmed that hydroxyl and imino groups were absent. By the action of methyl iodide, a quaternary salt was obtained as a colourless gum. The nitrogen was therefore tertiary and present in a single ring.

The u.v. spectrum of himbacine showed only strong end-absorption in the region 220–260 mμ (log ε 2·75—1·5). It was therefore non-aromatic and contained no conjugated system. The end absorption was presumably largely due to the nitrogen (Leonard and Locke 1955).

The i.r. spectrum of the alkaloid showed a band at 980 cm^{-1} (carbon disulphide) indicating the presence of a *trans*-disubstituted double bond. Himbacine was not readily attacked by ozone nor was it readily hydrogenated. At 3–4 atm in glacial acetic acid with platinum oxide catalyst, however, crystalline dihydrohimbacine, $C_{22}H_{37}O_2N$, was obtained, in the i.r. spectrum of which a band at 980 cm^{-1} was absent. Attempts to confirm the presence of an olefinic link by titration with perbenzoic acid were abandoned when reproducible results could not be obtained.

That two oxygen atoms were present in a γ-lactone system was indicated by bands at 1770 cm^{-1} (chloroform) and 1778 cm^{-1} (Nujol) (see also Cocker *et al.* 1953; Stenlake and Williams 1955; Deuel and Geissman 1957). Moreover, since the crystalline potassium salt obtained by boiling the alkaloid with concentrated aqueous ethanolic potassium hydroxide reverted to the alkaloid, on treatment with water or ethanol, the ring was *cis*-fused, and an enol ester group was absent (Angyal and Mills 1952; Newman and van der Werf 1945; Kuehl, Linstead, and Orkin 1950; Brewster and Kucera 1955).

Reduction of himbacine with lithium aluminium hydride yielded the expected "diol", $C_{22}H_{39}O_2N$, a non-crystalline substance, which was characterized as its methiodide. With hot dilute acid the diol gave an almost quantitative yield of the crystalline "anhydrodiol", $C_{22}H_{37}ON$, which on hydrogenation afforded the

amorphous " dihydroanhydrodiol ", $C_{22}H_{39}ON$, characterized as its methiodide. This substance was also prepared from dihydrohimbacine by lithium aluminium hydride reduction followed by treatment with hot dilute acid.

III. Dehydrogenation of Himbacine

Since himbacine had a comparatively high hydrogen content, its dehydrogenation was one of the first reactions to be examined. With palladium–charcoal at 260 °C, a fair yield of a crystalline base " dehydrohimbacine ", $C_{21}H_{29}O_2N$, was obtained. Since it contained no *N*-methyl group, its pK_a in 50% ethanol was 5·4 and its u.v. spectrum had a maximum at 265 mμ (log ε 3·65), the nitrogen was almost certainly present in a pyridine ring. This conclusion was supported by the i.r. spectrum which had bands at 1595 and 1580 cm^{-1} (pyridine ring) and 1760 cm^{-1} (saturated γ-lactone). The same substance was also obtained under the same conditions from dihydrohimbacine.

In the dehydrogenation of himbacine there was an overall loss of a methyl group and three hydrogen atoms. Now, in the formation of pyridine from *N*-methylpiperidine, the change requires the loss of a methyl group and five hydrogen atoms. Therefore, provided no carbon–carbon bands were broken during the dehydrogenation, the double bond of the alkaloid was incorporated in the aromatic ring of dehydrohimbacine or reduced during the reaction.

Selenium dehydrogenation of himbacine at 345–355 °C disrupted the molecule yielding methylamine, dimethyl diselenide (presumably) and two major fractions, a mixture of steam-volatile bases and a mixture of aromatic hydrocarbons. The basic fraction was approximately a 2 : 1 mixture of 2,6-dimethyl- and 2-ethyl-6-methylpyridines, which were isolated as their picrates, and no other base could be detected.

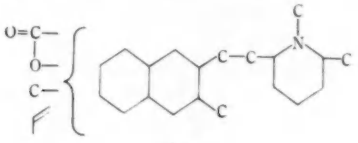
Chromatography of the hydrocarbon material on alumina yielded two fractions, both of which formed adducts with 1,3,5-trinitrobenzene. The major fraction on repeated recrystallization eventually yielded a pure substance which was identified as the TNB adduct of 2-ethyl-3-methylnaphthalene (m.p. 129 °C). Some difficulty was encountered in making this identification and distinguishing it from the TNB adduct of 2,3-diethylnaphthalene (m.p. 127 °C). Neither the analytical figures nor the melting points were sufficiently different to allow a decision to be made, the mixed melting point was undepressed, the u.v. spectra were identical, and the i.r. spectra showed only minor differences. The identification was made with certainty, however, by X-ray powder crystallography.

The second hydrocarbon product was obtained in only minute yield. It gave an orange-red TNB adduct, m.p. 182 °C, the analysis of which corresponded to a $C_{19}H_{16}$ compound and its u.v. spectrum readily identified it as a pyrene derivative. Subsequent work aimed at its synthesis (Moyle and Ritchie 1958) has shown, however, that it was probably a mixture in spite of its sharp and constant melting point.

In an experiment designed to place the carboxyl end of the lactone ring, the diol was also dehydrogenated with selenium. The same products as described above were obtained with only slight variations in the yields. It was therefore concluded that the carboxyl group was attached to a tertiary carbon atom.

To determine whether either of the ethyl groups of 2-ethyl-6-methylpyridine and 2-ethyl-3-methylnaphthalene was present as terminal groups in himbacine, the alkaloid was oxidized with chromic acid (Aycock, Eisenbraun, and McElvain 1954), and the volatile acids identified by paper chromatography (Lindqvist and Storgards 1953). Acetic acid, but not propionic acid, was detected, thus indicating that the molecule did not contain a terminal ethyl group. It is known that such groups do give rise to propionic acid on chromic acid oxidation (Bickel, Karrer, and Schmid 1955).

The above findings appeared to be most readily interpreted on the basis of structure (I), which explained the formation of the dehydrogenation products, including the pyrene, which could have arisen by elimination of methylamine followed by a double cyclization of the alkyl chain thus formed. It was adopted as a working hypothesis, although several objections against it could be raised.



(I)

IV. Exhaustive Methylations

A milder method of degradation was sought in exhaustive methylation. Himbacine methiodide was resistant to the action of potassium hydroxide in hot ethylene glycol. Attention was then turned to the methiodide of the dihydroanhydrodiol because the absence of hydroxyl groups and an ethylenic link promised a more straightforward degradation. When heated with 10% glycolic potassium hydroxide a crystalline "methine-I", $C_{23}H_{41}ON$, was obtained in high yield. Its i.r. spectrum revealed the presence of a vinyl bond (bands at 3100, 1647, 987, and 927 cm^{-1}, Nujol) which was confirmed by the production of formaldehyde on ozonolysis.

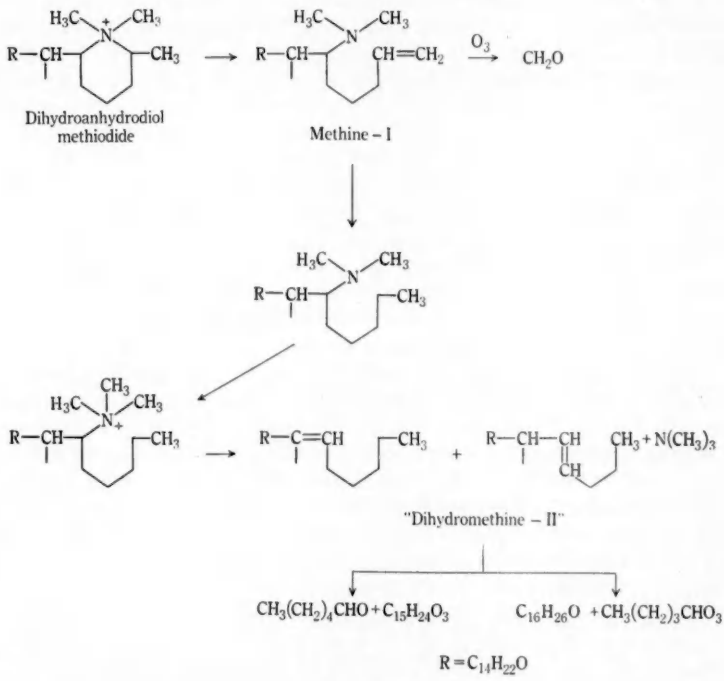
Catalytic hydrogenation gave crystalline "dihydromethine-I", $C_{23}H_{43}ON$, the methiodide of which yielded only 27% of nitrogen-free material on Hofmann degradation, the remainder reverting to dihydromethine-I. Abnormal partial or complete failure of Hofmann degradation has been previously recorded (Gellert 1956). The yield was unaltered by varying the concentration of potassium hydroxide. The elimination of trimethylamine was confirmed by isolation of its picrate.

The non-crystalline "dihydromethine-II" was oxidized by hydroxylation with osmium tetroxide followed by fission with periodic acid. The volatile aldehyde fraction was a mixture of approximately equal amounts of pentanal and hexanal, the DNP's of which were isolated.

This series of reactions can only be interpreted in the way shown in Flow Sheet 1. In the formation of methine-I, the Hofmann rule was followed, no

trace of acetaldehyde being detected on ozonolysis. The production of pentanal and hexanal from the dihydromethine-II showed that this material was a mixture of isomeric methines in approximately equal amounts.

A small portion of the non-volatile fraction from the oxidation was converted to its DNP, from which a single compound was eventually obtained, which analysed for the DNP of a $C_{15}H_{24}O_2$ aldehyde. It evidently arose from that dihydromethine-II from which hexanal was formed. Its u.v. spectrum ($\lambda_{max.}$ 359 mμ, log ε 4·2) showed it to be the DNP of a saturated carbonyl compound.



Flow Sheet 1

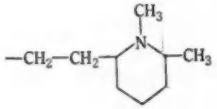
The remainder of the non-volatile fraction was oxidized with alkaline silver oxide to give a mixture of acids, which was separated into acid " A ", $C_{16}H_{26}O_3$, and acid " B ", $C_{15}H_{24}O_3$. Acid A was derived from the same methine as pentanal, and acid B along with hexanal from the other methine.

That the acid A was the next higher homologue of acid B was demonstrated by a Barbier-Wieland degradation. Next, a similar degradation of acid B was attempted. An unexpected result from the reaction of its methyl ester with excess phenylmagnesium bromide, was the formation in good yield of a compound, $C_{21}H_{28}O_2$, which had carbonyl absorption in the i.r. at 1680 cm^{-1} (Nujol). It

was therefore a phenyl ketone formed by reaction of the ester with only one equivalent of the Grignard reagent. Such a reaction is not commonly encountered since it is claimed that the second step of the reaction takes place more rapidly than the first (Gallagher, Long, and Marshall 1946; Kharasch and Reinmuth 1954).

The phenyl ketone on treatment with the more reactive phenyl-lithium gave a carbinol, from which an acid, $C_{14}H_{22}O_3$, was obtained in the usual way. It had the light absorption properties (1720 cm^{-1} Nujol; $\lambda_{max.}$ 220 mμ, log ε 2·56) of a saturated carboxylic acid and the absence of an isolated double bond was confirmed by its failure to give a colour with tetranitromethane.

This series of reactions showed conclusively that the two ring systems of himbacine were joined by a simple unbranched carbon bridge which contained at least two (and probably only two) carbon atoms, and that in dihydrohimbacine, structure (II) was present.



(II)

In the hope of determining the position of the double bond the methiodide of the anhydrodiol was then degraded. The "methine-I" obtained by the method used above was separated into three fractions. All were gums, but formed crystalline methiodides.

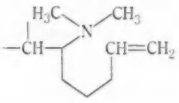
The first fraction showed end absorption in the u.v. ($\lambda_{max.}$ 216 mμ, log ε 3·12) similar to that of the anhydrodiol. Its methiodide was isomeric with, but clearly different from, the anhydrodiol methiodide. It was named "isoanhydrodiol" and presumably was formed by isomerization involving the double bond or one of the two asymmetric centres of the piperidine ring (cf. Gellert 1956).

The second fraction, showing only end absorption in the u.v., yielded a methiodide which had the spectrum of a saturated quaternary ammonium iodide ($\lambda_{max.}$ 221 mμ, log ε 4·17). It analysed for the methiodide of a methine base and has been named "methine-I methiodide".

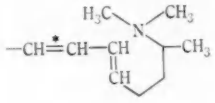
The third fraction showed a maximum in the u.v. at 234 mμ (log ε 4·42) and thus was a conjugated diene. Its methiodide showed maxima at 226 mμ (log ε 4·42) and 234 mμ (log ε 4·42), the first being due to the quaternary nitrogen and the second to the conjugated diene. This compound also analysed for the methiodide of a methine base and therefore was named "isomethine-I methiodide".

Considering the results of the previous Hofmann degradation, the present methine-I must be represented by (III) and the isomethine-I by (IV) or (V). To distinguish between them, the base was oxidized with permanganate in acetone. The acidic fraction yielded the $C_{14}H_{22}O_3$ acid, thus showing that the compound was represented by (IV).

There was also produced in low yield a neutral substance, $C_{13}H_{20}O_2$, which formed a DNP. Its light absorption properties ($\lambda_{max.}$ 288 mμ, log ε 1·49; 1705 cm^{-1} (carbon disulphide)) showed it to be a saturated ketone. It gave no colour with tetranitromethane and was therefore a saturated ketone containing two rings (excluding the tetrahydrofuran ring). Moreover, since the i.r. spectrum showed that the carbonyl group was not present in a strained ring, the ring to



(III)



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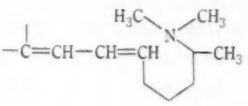
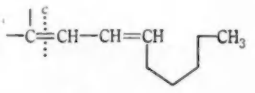


(V)

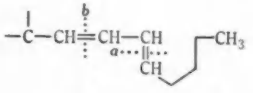
which the carbon bridge was attached was six-membered or greater. Since a naphthalene was formed on dehydrogenation of the alkaloid, a six-membered ring was favoured. One explanation of the formation of this ketone was that a small amount of the isomer (V) was present. Another possibility was that the $C_{14}H_{22}O_3$ acid had been further oxidized thus : $>CH-COOH \rightarrow >C=O$. Oxidations of this type are known to occur (Büchi and Goldman 1957).

The isolation of a compound containing structure (IV) did not necessarily place the double bond of the alkaloid in the position marked with an asterisk, since under the conditions of the reaction it could have moved into conjugation with the one introduced.

In the catalytic hydrogenation of the methine-I only one double bond was reduced giving crystalline dihydromethine-I, which retained the *trans*-ethylenic linkage (band at 980 cm^{-1}). Hofmann degradation of its methiodide led to the formation of a nitrogen-free oil, " dihydromethine-II " and the regeneration of some dihydromethine-I. The u.v. spectrum of the dihydromethine-II showed maxima at 236 mμ (log ε 4·31) and 242 mμ (log ε 4·28), indicating that it was a mixture of two conjugated dienes. Ozonolysis again yielded hexanal and pentanal, and therefore the two methines contained the partial structures (VI) and (VII).



(VI)

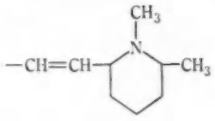


(VII)

The non-volatile fraction from the ozonolysis yielded on chromatography a fraction from which a crystalline DNP was obtained. It was the derivative of a $C_{16}H_{24}O_2$ aldehyde and since its u.v. spectrum had a maximum at 375 mμ (log ε 4·47), it was the DNP of an αβ-unsaturated aldehyde. The presence of an unsaturated compound in the ozonolysed mixture was not expected but its isolation was added proof for the presence in the dihydromethine-II mixture of a

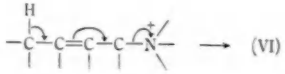
compound containing structure (VII), the aldehyde having been formed by oxidation at the point (*a*).

Because of the partial ozonolysis, oxidation was completed with permanganate in acetone to yield the $C_{14}H_{22}O_3$ acid and the $C_{13}H_{20}O_2$ ketone, by cleavage at (*b*) and (*c*) respectively. This series of reactions proved that the ethylenic linkage of himbacine was not present in the piperidine ring and since the i.r. spectrum of the alkaloid showed that the double bond was *trans*- and disubstituted, it appeared that the only position it could occupy was as shown in structure (VIII). This grouping accounted for all the known facts,



(VIII)

including the formation of a dihydromethine-II of structure (VI), which could arise by vinylogous elimination :



(VI)

V. Oxidation Experiments

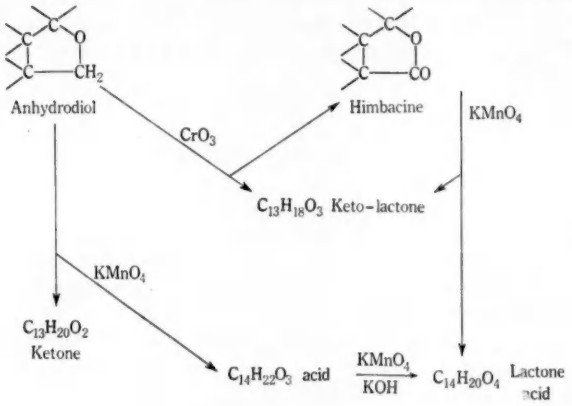
To confirm the position of the double bond, a number of oxidation experiments were carried out. The alkaloid was not readily attacked by ozone, nor could a glycol be obtained by osmium tetroxide hydroxylation. Similarly, the anhydrodiol gave intractable mixtures on attempted hydroxylation with osmium tetroxide or performic acid. With perphthalic acid, the anhydrodiol gave a crystalline compound, $C_{22}H_{37}O_2N$, which was proven to be the *N*-oxide by regeneration of the base on reduction.

Oxidation of the anhydrodiol with permanganate gave significant results only under carefully controlled conditions. There was obtained a moderate yield of the $C_{14}H_{22}O_3$ acid, thus proving the position of the double bond to be placed as in (VIII). The $C_{13}H_{20}O_3$ ketone was also isolated in low yield, presumably being formed by further oxidation of the acid as postulated above.

A similar oxidation of himbacine gave the corresponding acid, $C_{14}H_{20}O_4$. The i.r. spectrum of the substance in chloroform had a band at 1770 cm^{-1}, confirming the presence of a saturated γ-lactone and its weak end-absorption in the u.v. and failure to react with tetranitromethane showed it to be a saturated bicyclic compound (excluding the lactone ring). The neutral fraction from the oxidation gave a low yield of a substance, $C_{13}H_{18}O_3$, which proved to be a keto-lactone. It formed a DNP and its i.r. spectrum showed the presence of a saturated γ-lactone (1783 cm^{-1}, carbon disulphide) and a strain-free saturated ketone (1710 cm^{-1}, carbon disulphide). Moreover, the weak end-absorption at 220 mμ

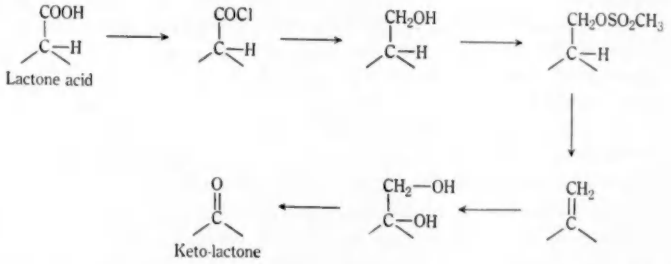
(log ε 1·98) and a maximum at 292 mμ (log ε 1·33) confirmed that it was a saturated ketone.

A slightly higher yield of the keto-lactone was obtained on oxidation of himbacine with chromic anhydride in warm acetic acid, but none of the related acid could be isolated. A similar oxidation of the anhydrodiol gave an unexpected but useful result. Himbacine was isolated, being formed by oxidation



Flow Sheet 2

of the tetrahydrofuran ring to a γ-lactone. This result showed conclusively that in the formation of the anhydrodiol from the alkaloid via the diol, no rearrangement of the carbon skeleton had occurred. From the oxidation, a small yield of the keto-lactone was also obtained. The inter-relationships established by these oxidation experiments are summarized in Flow Sheet 2.



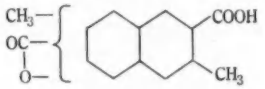
Flow Sheet 3

Although the C_{13} ketones were almost certainly formed by further oxidation of the C_{14} acids, an attempt was made to prove this point by direct alkaline permanganate oxidation (Büchi and Goldman 1957). However, the $C_{14}H_{22}O_3$ acid yielded no neutral material, instead oxidation of the tetrahydrofuran ring occurred, giving the $C_{14}H_{20}O_4$ lactone acid. A similar oxidation of the latter also gave no neutral material.

A conversion of the lactone acid to the keto-lactone was, however, achieved by a less direct series of reactions. By the action of thionyl chloride the lactone acid afforded the acid chloride which was reduced by sodium borohydride to the primary alcohol. The methane sulphonate of this substance was heated under reflux with collidine to yield the ene-lactone, which by successive hydroxylation with osmium tetroxide and cleavage with periodate gave the keto-lactone. The transformations are shown in Flow Sheet 3.

VI. The Chemistry of the C_{14} Compounds

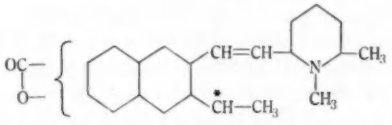
On the basis of the proposed skeleton (I) for himbacine, the $C_{14}H_{20}O_4$ lactone acid was represented by (IX). One fact which did not favour such a structure was the consistently low *C*-methyl determinations on the $C_{14}H_{22}O_3$ and $C_{15}H_{24}O_3$ acids, the values being 0·81 and 0·84 respectively. Even allowing for a low yield of acetic acid from an angular methyl group, the figures would have been expected to be slightly greater than one. To test the correctness of a structure



(IX)

of this type, the lactone acid was dehydrogenated with palladium-charcoal at 320–330 °C. Instead of the expected 2-methylnaphthalene which would have arisen by decarboxylation and elimination of the angular methyl group, there was obtained 2-ethylnaphthalene.

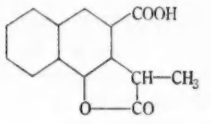
This result showed that two of the earlier assumptions were incorrect, namely that the ethyl groups of 2-ethyl-3-methylpyridine and 2-ethyl-3-methylnaphthalene were derived from the same structure in the molecule, and that himbacine contained an angular methyl group because together, 2-ethylnaphthalene, the lactone ring, and the carboxyl group, accounted for all the carbon atoms of the C_{14} acid. It now appeared that the methyl group of the 2-ethyl-3-methylnaphthalene marked the point of attachment of the remainder of the molecule and therefore a structure of the type (X) was proposed for the alkaloid.



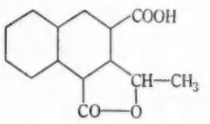
(X)

Previously, because selenium dehydrogenation of the diol also gave 2-ethyl-3-methylnaphthalene, it had been proposed that the carboxyl end of the lactone ring was attached to a tertiary carbon. Now, since himbacine did not contain a *C*-ethyl group, in a structure such as (X), one end of the lactone ring must have been attached to the carbon atom marked with an asterisk. Also, since the point of attachment of the carbon bridge must be at a secondary carbon to permit

the formation of the C_{13} ketones, there was only one position to which the other end of the lactone ring could be joined. On this basis the lactone acid was either (XI) or (XII). In postulating these structures it was implied that elimination of a non-angular hydroxymethyl group had occurred in the dehydrogenation of the diol. Such an explanation was supported by the finding of Newman and O'Leary (1946) that 1-hydroxymethyltetralin gave mainly naphthalene on dehydrogenation with palladium–charcoal.

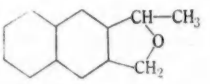


(XI)

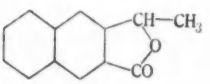


(XII)

In the hope of obtaining a naphthalene derivative with a methyl group derived from the carboxyl end of the lactone ring, the $C_{14}H_{22}O_3$ acid, obtained from the anhydrodiol, was dehydrogenated with palladium–charcoal. Unexpectedly, the product was 2-ethyl-3-methylnaphthalene. Its formation led to the conclusion that structure (XIII) was present in the anhydrodiol and that therefore himbacine contained structure (XIV).



(XIII)



(XIV)

The first assumption that the ethyl group and later that the methyl group of the 2-ethyl-3-methylnaphthalene formed by selenium dehydrogenation of himbacine, marked the point of attachment of the rest of the molecule, had thus both been shown to be incorrect. It was now clear that this naphthalene derivative was formed by reduction of the carboxyl of the lactone and complete elimination of the hydrocarbon bridge. The point of attachment of the other end of the lactone was placed as in (XIV) because of the restriction that this fragment of the molecule contained a *C*-methyl and not a *C*-ethyl group.

It should be noted that similar cases of the reduction of carboxyl to methyl groups have been encountered (Thiele and Trautmann 1935 ; Thiele and Windaus 1935 ; Ruzicka 1936), the most notable being the formation of 2,3-dimethylnaphthalene on selenium dehydrogenation of a dihydro-derivative of an adduct formed from maleic anhydride and vitamin D_2. The reduction did not occur on dehydrogenation by palladium–charcoal. Also, several examples of the elimination of non-angular alkyl groups in the aromatization of tetralin derivatives have been described by Cocker, Cross, and McCormick (1952).

Considering the structures (VIII) and (XIV) proposed for the two " halves " of himbacine, it seemed that the point of attachment of (VIII) to (XIV) was all that remained to be shown. However, it must be stated that the presence of

a decalin nucleus had not been conclusively proven because of the uncertainty which is always associated with dehydrogenations. Nevertheless, the partial structure (XIV) seemed to be the most likely and shall be used in the following discussion.

Since each of the C_{14} acids contained a carboxyl group which marked the point of attachment of the rest of the molecule, it appeared that a suitably reduced compound on dehydrogenation at the lower temperatures required for palladium–charcoal or sulphur, might give a known or synthesizable C_{14} naphthalene. The most suitable compound was the $C_{14}H_{22}O_3$ acid. Reduction of its methyl ester with lithium aluminium hydride gave the crystalline alcohol, $C_{14}H_{24}O_2$, which when heated with palladium–charcoal at 320–330 °C, gave a mixture of naphthalenes from which pure TNB adducts could not be isolated. Apparently partial elimination of the hydroxymethyl group had occurred.

To overcome this difficulty, the alcohol was converted to its *p*-toluenesulphonate which was reduced to give a non-crystalline deoxy compound. However, palladium–charcoal dehydrogenation again yielded a mixture of naphthalenes from which pure TNB adducts could not be isolated. Analysis of the mixture indicated that it was composed of C_{13} and C_{14} naphthalenes. An attempt to effect dehydrogenation with sulphur at 210–230 °C, at which temperature elimination does not occur (Cocker *et al.* 1953), gave no naphthalenes.

During this work, the preparation of the $C_{14}H_{24}O_2$ alcohol was attempted from the more readily accessible $C_{14}H_{20}O_4$ acid lactone. Reduction of its methyl ester to a non-crystalline triol with lithium aluminium hydride, followed by dehydration with hot dilute acid, gave an isomeric alcohol. The nature of this substance was not examined further, but its formation did suggest a 1,2,3-arrangement of groups in the decalin nucleus.

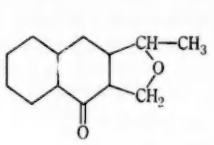
VII. The Chemistry of the C_{13} Ketones

Further support for a 1,2,3-arrangement was sought in the behaviour of two C_{13} ketones. The $C_{13}H_{20}O_2$ ketone failed to give either a piperonylidene derivative or a positive Zimmermann reaction, and therefore did not contain an unhindered active methylene group. Hence its structure was either (XV) or (XVI), and the structure of the keto-lactone correspondingly either (XVII) or (XVIII). Since the keto-lactone did not give a positive ferric test it was not a β-keto ester and was therefore (XVIII).

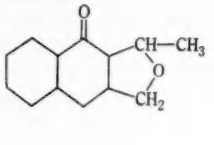
Further and conclusive evidence that (XVIII) was correct was obtained from the behaviour of the substance in ethanolic potassium hydroxide, which was studied spectroscopically. Whereas its spectrum in ethanol showed absorption due to a saturated aliphatic ketone ($\lambda_{max.}$ 220 mμ, log ε 1·98; $\lambda_{max.}$ 292 mμ, log ε 1·33), in ethanol containing 0·2% potassium hydroxide, it had a spectrum typical of an αβ-unsaturated ketone ($\lambda_{max.}$ 248 mμ, log ε 3·45; $\lambda_{max.}$ 318 mμ, log ε 1·91) which was unchanged on acidification with acetic acid. Such changes are well known (e.g. Barton and de Mayo 1957), the mechanism being as in Flow Sheet 4.

The αβ-unsaturated ketone which was obtained as a gum, on ozonolysis afforded acetaldehyde, isolated as its DNP, and an acidic gum. The latter on

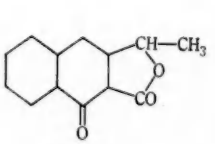
heating at 120–150 °C evolved carbon dioxide and yielded an acid, $C_{10}H_{16}O_4$, m.p. 170 °C, $[\alpha]_D$ +47°, which if structure (XVIII) were correct for the keto-lactone, would have been (+)-β-[*trans*-2-carboxycyclohexyl]propionic acid (XIX). The racemic *trans*-acid, m.p. 143 °C, which has been reported by Hückel,



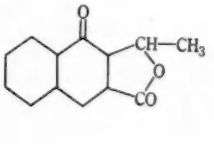
(XV)



(XVI)

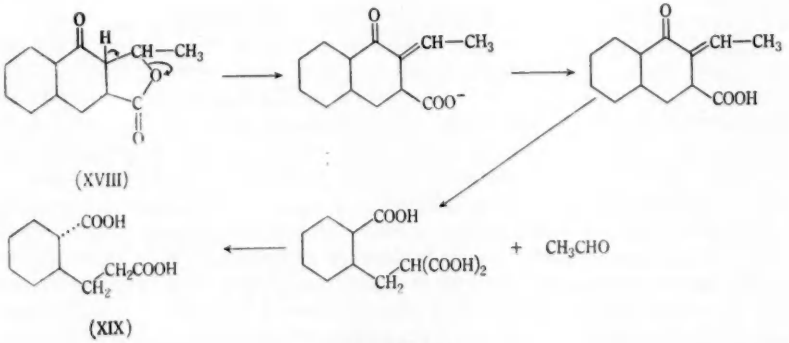


(XVII)



(XVIII)

Reverey, and Windaus (1923), was synthesized by a modification of their method and obtained with m.p. 140 °C. When mixed with the natural material the melting point lay between those of the two compounds. Because of the low solubility of the two acids in chloroform, carbon tetrachloride, and carbon

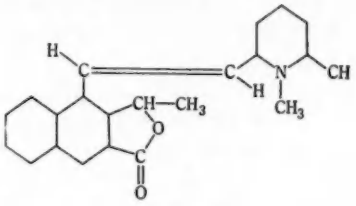


Flow Sheet 4

disulphide, their i.r. spectra in solution could not be profitably compared. However, their spectra in Nujol were identical and therefore the "natural" acid did in fact have structure (XIX). The conversion of the αβ-unsaturated acid to (XIX) may then be represented as in Flow Sheet 4. It should be noted that the degradation of (XVIII) to (XIX) establishes the presence of a decalin

residue in himbacine and excludes the possibility of any structures containing five- or seven-membered rings. The final isolation of a *trans*-acid in this degradation did not prove that the decalin residue in himbacine was *trans*, since if a *cis*-decalin residue had been present isomerization would have occurred when (XVIII) was treated with alkali.

The evidence presented above establishes that himbacine has structure (XX). The double bond is known to be *trans*, the lactone ring is probably *cis*-fused, and the decalin residue is thought to be *trans*.



(XX)

These conclusions have been confirmed, quite independently, by a full X-ray crystallographic analysis of himbacine hydrobromide carried out by Fridrichsons and Mathieson (1960, 1961). In addition, they have succeeded in deriving the absolute stereochemistry of the alkaloid.

VIII. The Structure of Himbeline

Himbeline was previously (Brown *et al.* 1956) thought to be isomeric with himbacine and assigned the formula $C_{22}H_{35}O_2N$, but a subsequent analysis showed the absence of an *N*-methyl group, and also the i.r. spectrum the presence of an imino group (band at 3310 cm^{-1}). On re-examination of the question, it was found that the analytical figures for the free base fitted equally well the formula $C_{21}H_{33}O_2N$. That this was the correct formula was readily demonstrated by methylating himbeline with formaldehyde–formic acid to himbacine. Himbeline was therefore *N*-demethylhimbacine.

It readily yielded an acetyl and a methanesulphonyl derivative, but unexpectedly these derivatives were not hydrolysed back to himbeline under the usual conditions, which indicated the operation of a subtle steric effect. This effect, which is being further examined, is presumably connected with the unreactivity of the double bond of himbacine. By contrast, the double bond of himbeline was readily hydrogenated under normal conditions to yield dihydrohimbeline, was readily oxidized by permanganate giving the $C_{14}H_{20}O_4$ lactone acid, and was attacked by performic acid to yield himbeline epoxide and himbeline glycol.

IX. The Structure of Himandravine

Himandravine, $C_{21}H_{33}O_2N$, like himbeline, with which it was isomeric, contained no *N*-methyl group. Its i.r. spectrum showed the presence of an imino group, a saturated γ-lactone group, and a *trans*-disubstituted double bond (bands at 3320, 1765, 976 cm^{-1} in Nujol).

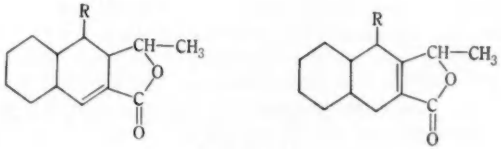
With formaldehyde–formic acid it gave *N*-methylhimandravine, and on catalytic hydrogenation readily afforded dihydrohimandravine, which yielded *N*-methyldihydrohimandravine by methylation. However, catalytic hydrogenation of *N*-methylhimandravine under the usual conditions did not occur, thus recalling the behaviour of himbacine. Again, like himbeline, himandravine was readily oxidized by permanganate to the $C_{14}H_{20}O_4$ acid lactone.

These results suggested that himandravine was a stereoisomer of himbeline. This was proved to be so by palladium–charcoal dehydrogenation which gave dehydrohimbacine in good yield. Himandravine therefore differs from himbeline only by having a different configuration at one or both of the asymmetric centres in the piperidine ring.

X. The Structure of Himgravine

With Sylvia D. Binns*

Himgravine, $C_{22}H_{33}O_2N$, contained one *N*-methyl group and was a fairly strong base, having a pK_a of 9·3 (50% ethanol). Its spectral properties (bands at 1754, 1684 cm^{-1}; $\lambda_{max.}$ 218 mμ, log ε 4·0) revealed the presence of an αβ-unsaturated γ-lactone ring. On catalytic hydrogenation it gave a good yield of himbacine, from which it followed that himgravine was either (XXI) or (XXII).



R = $C_9H_{16}N$

(XXI) (XXII)

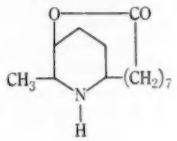
A decision in favour of the former structure has been reached from a study of the n.m.r. spectrum of the alkaloid (Abraham and Bernstein 1961).

XI. Structural Relationships

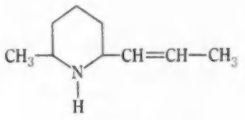
Himbacine, himbeline, himandravine, and himgravine clearly constitute a new group of alkaloids. It seems certain that they are neither isoprenoid nor related to the large *Lobelia* group. However, there are three other bases, carpaine (XXIII), pinidine (XXIV), and muscopyridine (XXV) which are also 2,6-disubstituted pyridine derivatives. The three consist of an unbranched carbon chain into which a nitrogen atom has been fitted and schemes based on an acetate–propionate type of hypothesis have been suggested for their biogenesis (cf. Biemann, Büchi, and Walker 1957). In a similar manner the biogenesis of himbacine may be represented by similar schemes which are summarized in (XXVI) and (XXVII). Objections to this hypothesis are immediately obvious

* Department of Organic Chemistry, University of Sydney.

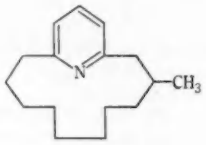
and, in addition, the decalin moiety certainly could be derived from shikimic acid, but further speculation at this stage would be fruitless. It is hoped that studies of the other alkaloids of the species, now in progress, will provide evidence on the question of their biogenesis.



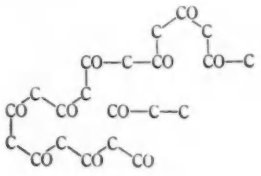
(XXIII)



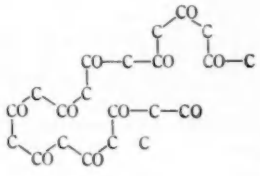
(XXIV)



(XXV)



(XXVI)



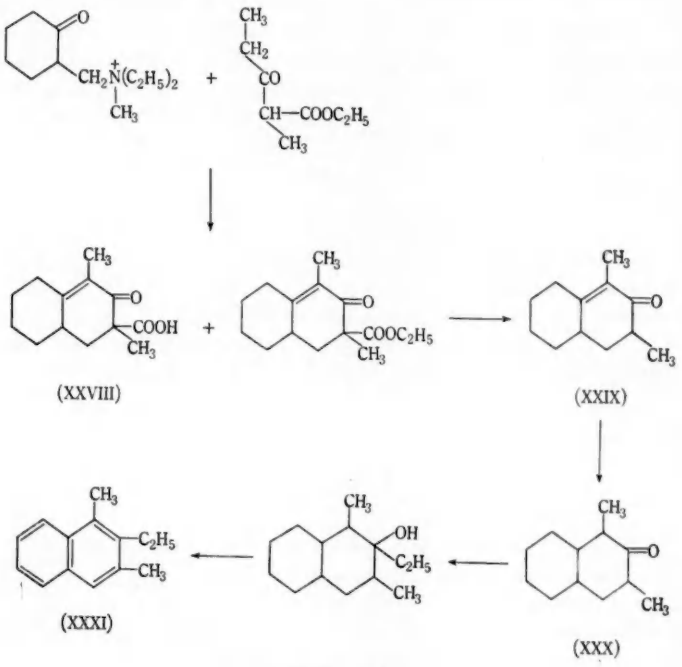
(XXVII)

XII. The Synthesis of 1,3-Dimethyl-2-ethylnaphthalene

When experiments on the dehydrogenation of the $C_{14}H_{24}O_2$ alcohol and the related deoxy compound were in progress it was thought to be desirable to have on hand a specimen of the expected product, 1,3-dimethyl-2-ethylnaphthalene.

The synthesis was accomplished by an extension of the method of du Feu, McQuillin, and Robinson (1937) using the improved conditions of Shunk and Wilds (1943), and is summarized in Flow Sheet 5. The reaction between the sodio derivative of ethyl α-propionylpropionate and 2-diethylaminoethylcyclohexanone methiodide in methanol–benzene yielded two fractions. The first, a crystalline acid, $C_{13}H_{18}O_3$, obtained in low yield, was readily decarboxylated and had the u.v. spectrum of an αβ-unsaturated ketone ($\lambda_{max.}$ 249 mμ, log ε 4·14). This substance was therefore 1,3-dimethyl-2-oxo-3-carboxy-2,3,4,5,6,7,8,10-octahydronaphthalene (XXVIII) for which the calculated absorption maximum was 254 mμ. The other, the major fraction, was a neutral oil which was converted to 1,3-dimethyl-2-oxo-2,3,4,5,6,7,8,10-octahydronaphthalene (XXIX) ($\lambda_{max.}$ 248 mμ, log ε 4·07), characterized by its crimson DNP derivative. Hydrogenation of (XXIX) gave the saturated ketone (XXX) also characterized by its orange DNP derivative. Treatment of (XXX) with ethylmagnesium bromide gave the tertiary carbinol which, without purification, was dehydrogenated with palladium–charcoal at 280–300 °C to the required 1,3-dimethyl-2-ethylnaphthalene (XXXI), characterized by its yellow TNB adduct.

The TNB adducts of 2-isopropylnaphthalene (Bergmann and Weizmann 1944) and 2,5-dimethyl-3-ethylnaphthalene (Funakoshi *et al.* 1957) were also prepared as reference compounds.



Flow Sheet 5

XIII. Experimental

Melting points are uncorrected. Light petroleum refers to the fraction, b.p. 60–90 °C, unless otherwise stated. Ultraviolet spectra were measured in purified ethanol on a Hilger (Uvispek) Model 6 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer Model 21B spectrophotometer equipped with sodium chloride optics. Optical rotations were measured on chloroform solutions unless otherwise stated. Analyses were performed by Miss B. Stevenson of this Department and by the C.S.I.R.O. Microanalytical Laboratory, Melbourne. For the formation of DNP's, a 2% solution of the reagent in 30% perchloric acid was used. The alumina and acid-washed alumina used for chromatography had Brockmann activity II. " Worked up in the usual manner " means that the solution was extracted with ether, the extract dried over anhydrous sodium sulphate, and the solvent removed.

Himbacine

(*a*) *Himbacine Hydrobromide.*—The *substance* crystallized from acetone in colourless, rectangular prisms, m.p. 192–193 °C (Found (after drying for 12 hr at 20 °C/20 mm): C, 60·8; H, 8·6%. Calc. for $C_{22}H_{36}O_2NBr.\frac{1}{2}H_2O$: C, 60·7; H, 8·5%). By varying the conditions of drying, products of greater or less degree of hydration could be obtained.

(*b*) *Dihydrohimbacine.*—The alkaloid (0·4 g) in glacial acetic acid (20 ml) was hydrogenated at 3–4 atm and room temperature for 16 hr, using platinum oxide (0·2 g). The catalyst was removed, the filtrate basified with ammonia and worked up in the usual manner. The *product* crystallized from hexane in colourless prisms, m.p. 80–81 °C, $[\alpha]_D^{14}$ +88° (*c*, 1·04) (Found: C, 76·0; H, 10·7%. Calc. for $C_{22}H_{37}O_2N$: C, 76·0; H, 10·7%).

The *methiodide*, prepared in ethanol with excess methyl iodide, crystallized from ethanol–ether in colourless prisms, m.p. 216–217 °C (Found: C, 56·5; H, 8·1%. Calc. for $C_{23}H_{40}O_2NI$: C, 56·4; H, 8·2%).

(*c*) *Hydrolysis of the Lactone Ring.*—When refluxed for 0·5 hr with 15% aqueous KOH (20 ml), himbacine (0·3 g) appeared unchanged. On the addition of ethanol (5 ml), solution was effected. On slowly distilling off the ethanol after about 0·2 hr, a crystalline precipitate separated from the hot solution. The precipitate, which was collected and washed with a little water, did not melt below 300 °C. When crystallization from water was attempted, it dissolved slowly, reprecipitating himbacine. The potassium salt when heated with ethanol, also regenerated himbacine.

(*d*) *Lithium Aluminium Hydride Reduction.*—A solution of himbacine (2·0 g) in tetrahydrofuran (50 ml) was added in one portion to a suspension of lithium aluminium hydride (0·14 g) in tetrahydrofuran (50 ml) at 0 °C. After 2 hr, the mixture was allowed to warm to room temperature and then kept overnight. Excess reagent was decomposed with water, the precipitated aluminium hydroxide dissolved by 5% HCl and then cold 40% NaOH added until the precipitate just redissolved. On working up in the usual manner, the diol was obtained as a clear gum (2·0 g) which did not crystallize. It was dissolved in ethanol (10 ml) and methyl iodide (1·5 ml) added. After warming for a short time, the *methiodide* was precipitated by ether. It crystallized in small colourless needles (2·2 g), m.p. 216–217 °C (Found: C, 56·2; H, 8·4%. Calc. for $C_{23}H_{42}O_2NI$: C, 56·2; H, 8·6%).

(*e*) *The Anhydrodiol.*—A solution of the diol (5·5 g) in 4% H_2SO_4 (25 ml) was heated on the steam-bath for 4 hr and then kept overnight. On basifying and working up as usual, a *solid* (5·0 g) was obtained which crystallized from hexane in colourless needles, m.p. 123 °C (Found: C, 79·7; H, 11·2%. Calc. for $C_{23}H_{37}ON$: C, 79·7; H, 11·3%).

The *methiodide*, obtained by treating the base with excess methyl iodide, crystallized from ethanol–ether in colourless needles, m.p. 178–181 °C, $[\alpha]_D^{14}$ +49° (*c*, 1·00) (Found: C, 58·1; H, 8·6%. Calc. for $C_{24}H_{40}ONI$: C, 58·3; H, 8·5%).

(*f*) *Dihydroanhydrodiol.*—(i) Catalytic hydrogenation of the anhydrodiol as in (*b*) gave a colourless gum. On treatment with excess methyl iodide it yielded a *methiodide* which crystallized from ethanol–ether in colourless needles, m.p. 247–248 °C, $[\alpha]_D^{14}$ +38° (*c*, 0·80) (Found: C, 58·0; H, 8·9%. Calc. for $C_{24}H_{42}ONI$: C, 58·1; H, 8·9%).

(ii) Dihydrohimbacine (4·9 g) was allowed to stand overnight in dry ether (200 ml) containing lithium aluminium hydride (0·6 g). Excess reagent was decomposed by the addition of water and the ether removed. The residue was dissolved in 5% H_2SO_4 (80 ml) and the solution heated on a steam-bath for 3 hr. After cooling, the solution was basified and worked up in the usual manner to yield a gum from which a methiodide (5·8 g), identical with that obtained above, was prepared.

(*g*) *Dehydrohimbacine.*—(i) Himbacine (1·2 g) and 10% palladium–charcoal were heated together at 255–265 °C for 2·5 hr. The residue was exhausted with hot chloroform and the extract evaporated. The residual brown gum was dissolved in ether and the solution extracted with 5% HCl, then 5% NaOH, and finally washed with water.

During the acid extraction, insoluble material (0·15 g) separated. It was shaken with ammonia and ether, and the extract worked up as usual, but no individual substance could be isolated from the resulting dark gum.

The basic material recovered from the acid extract was dissolved in benzene–light petroleum (50 : 50) and chromatographed on alumina (10 g). The benzene–light petroleum eluate (50 : 50) gave a gum (30% yield) which crystallized from heptane in colourless needles, m.p. 141–142 °C, $[\alpha]_D^{14}$ +65·5° (*c*, 0·89) (Found: C, 77·1; H, 8·8; N, 4·2%; (*N*)CH_3, nil. Calc. for $C_{21}H_{29}O_2N$: C, 77·0; H, 8·9; N, 4·3%).

No crystalline material could be obtained from the other eluates.

The ether solution yielded a brown neutral gum (0·14 g) from which pure substances could not be isolated. No acidic material was present in the alkaline extract.

(ii) Dehydrogenation of dihydrohimbacine under the above conditions gave a slightly higher yield (36%) of dehydrohimbacine.

(*h*) *Selenium Dehydrogenations.*—(i) An intimate mixture of himbacine (10 g) and selenium (50 g) was placed in a 250 ml distilling flask to which was connected a short condenser with a receiver and a trap containing 5% HCl. Under passage of a slow stream of nitrogen, the mixture was heated at 290–300 °C for 0·3 hr. The dark liquid with an intolerable, penetrating odour, presumably dimethyl diselenide, which collected in the receiver, was discarded. The contents of the acid trap were evaporated to dryness, the residue dissolved in a little water and treated with aqueous lithium picrate. The precipitate (0·4 g) after several recrystallizations from aqueous ethanol, gave methylamine picrate, m.p. and mixed m.p. 215 °C (Found: C, 32·2; H, 2·9%. Calc. for $C_7H_8O_7N_4$: C, 32·3; H, 3·1%).

After attaching another receiver and acid trap, the dehydrogenation was continued at 345–355 °C for 14 hr. The trap and receiver were washed thoroughly with ether and 5% HCl. The phases were shaken and then separated to give a volatile basic fraction in acid solution and a volatile neutral fraction in ether.

The dark mass in the distilling flask was finely powdered and exhausted with chloroform. The solvent was removed and light petroleum added to the residue. After keeping for several days, the extract was filtered from dark selenium-containing material, and the filtrate extracted with 5% HCl, to yield a non-volatile neutral, and a non-volatile basic fraction.

The acid solution containing the non-volatile bases was made alkaline and extracted with ether. After drying and removing the solvent, a small amount of a dark viscous oil remained. All attempts to isolate pure substances from it were fruitless.

The solutions containing the volatile and non-volatile neutral fractions were combined and evaporated to dryness. The residue was dissolved in light petroleum and chromatographed on alumina ($\frac{3}{4} \times 18$ in.), the development of the chromatogram being conveniently followed with the aid of a u.v. lamp. Seventeen fractions each of about 100 ml were collected. The solvent was removed from each fraction, the residues dissolved in hot ethanol (10 ml), and the solutions treated with small amounts of solid 1,3,5 trinitrobenzene. On cooling, fractions 1–3 gave yellow crystalline adducts with m.p.'s from 105–115 °C (1·98 g), fractions 4–9 traces of the same product, and fractions 10–17 an orange crystalline adduct, m.p. 160 °C (0·09 g). The mother liquors from each fraction were treated again with TNB to ensure complete conversion to the adduct.

The yellow adduct on repeated recrystallization from ethanol gave pale yellow needles, m.p. 129 °C (Found: C, 59·2; H, 4·5; N, 11·7%. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·5; H, 4·4; N, 11·0%). It was identified as described above in Section III as the *TNB adduct of 2-ethyl-3-methylnaphthalene*. The mother liquors from the recrystallizations were combined, the hydrocarbon fraction regenerated and rechromatographed as before, but no adduct other than that of 2-ethyl-3-methylnaphthalene could be isolated.

The orange adduct after many recrystallizations from ethanol gave fine orange-red needles (0·011 g) of constant m.p. 182 °C (Found: C, 65·5; H, 4·5%. Calc. for $C_{25}H_{19}O_6N_3$: C, 65·6; H, 4·2%). The nature of this pyrene derivative has been discussed in Section III.

The acid solution containing the volatile bases was made strongly alkaline and steam distilled. The distillate was saturated with salt and extracted with ether. The extract was dried over NaOH and the ether carefully removed under reduced pressure, leaving an almost colourless liquid (1·6 g). Fractional crystallization of the picrate of this material was very tedious and only partially successful in effecting a separation. Better separation was achieved by slow and careful fractional distillation through a semimicro fractionating column. Six fractions, each of about 0·15 g, were collected, the seventh, about 0·5 g, remaining in the distilling flask. The first fraction gave a picrate, m.p. 150 °C, which was strongly depressed on admixture with the picrate of 2-methylpyridine. The picrates of fractions 2–6 melted between 140 and 131 °C. The picrates of all six fractions were combined and the material repeatedly recrystallized from methanol to yield dark yellow prisms, m.p. 159–160 °C, undepressed on admixture with authentic

2,6-dimethylpyridine picrate (m.p. 160 °C) (Found : C, 46·5 ; H, 3·9%. Calc. for $C_{12}H_{12}O_7N_4$: C, 46·4 ; H, 3·6%).

Fraction 7, the material undistilled, gave a picrate which crystallized from ethanol in yellow plates, m.p. 126–127 °C, undepressed by the authentic picrate of 2-ethyl-6-methylpyridine (m.p. 127 °C) (Found : C, 47·8 ; H, 4·0%. Calc. for $C_{14}H_{14}O_7N_4$: C, 48·0 ; H, 4·0%).

(ii) Dehydrogenation of the diol under the same conditions gave the same products in about the same yields.

(iii) 2-Ethyl-3-methylnaphthalene (Smith and Lo 1948) and 2,3-diethylnaphthalene (Arnold and Barnes 1944) were prepared by the general method of the former authors.

The TNB adduct of ethylmethylnaphthalene crystallized from ethanol in pale yellow needles, m.p. 129 °C (Found : C, 59·6 ; H, 4·6 , N, 11·1%. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·5 ; H, 4·4 ; N, 10·9%).

The *TNB adduct of 2,3-diethylnaphthalene* separated from ethanol as yellow needles, m.p. 127 °C (Found : C, 60·4 ; H, 4·7 ; N, 10·5%. Calc. for $C_{20}H_{19}O_6N_3$: C, 60·5 ; H, 4·8 ; N, 10·6%).

(*i*) *Exhaustive Methylation of the Dihydroanhydrodiol.*—(i) A solution of the methiodide (6·0 g) and KOH (4·0 g) in ethylene glycol (40 ml) was refluxed for 3 hr, during which time an oily layer separated. The mixture was diluted and extracted with ether. The ether solution was extracted with 5% HCl and the bases recovered as usual. The *methine-I* (4·0 g) crystallized from hexane in large colourless prisms, m.p. 55–57 °C, $[\alpha]_D^{14}$ +33° (*c*, 1·52) (Found : C, 79·2 ; H, 11·6%. Calc. for $C_{23}H_{41}ON$: C, 79·5 ; H, 11·9%). The *methiodide* crystallized from ethanol–ether in colourless plates, m.p. 170–171 °C (Found : C, 59·1 ; H, 9·2%. Calc. for $C_{24}H_{44}ONI$: C, 58·9 ; H, 9·1%).

On working up the mother liquors from the purification of methine-I by chromatography, a small amount (0·2 g) of the anhydrodiol was recovered.

(ii) A solution of the methine-I (0·5 g) in glacial acetic acid (10 ml) was ozonized at 10 °C for 1 hr. After the addition of zinc dust, water (30 ml) was added and the mixture partially distilled into DNP reagent. The precipitated DNP was extracted with benzene, the extract washed, dried, and the solvent removed, leaving an orange crystalline product (0·19 g, 63% yield of formaldehyde DNP). Purification by chromatography in benzene on acid-washed alumina (5·0 g) followed by crystallization from benzene–light petroleum gave formaldehyde DNP, m.p. and mixed m.p. 161–162 °C (Found : C, 40·3 ; H, 3·1 ; N, 26·6%. Calc. for $C_7H_6O_4N_4$: C, 40·0 ; H, 2·9 ; N, 26·7%).

A crystalline product could not be isolated from the original residual acid solution from the ozonolysis.

(iii) Catalytic hydrogenation of methine-I (0·5 g) in ethanol (20 ml) at atmospheric pressure using platinum oxide (0·05 g) yielded *dihydromethine-I*, which crystallized from ethanol in needles, m.p. 53–54 °C, $[\alpha]_D^{21}$ +32° (*c*, 1·18) (Found : C, 79·1 ; H, 12·3%. Calc. for $C_{23}H_{43}ON$: C, 79·2 ; H, 12·4%).

The *methiodide* crystallized from ethanol–ether in colourless plates, m.p. 181 °C (Found : C, 58·5 ; H, 9·4%. Calc. for $C_{24}H_{46}ONI$: C, 58·6 ; H, 9·4%).

(iv) The above methiodide (8·5 g) was refluxed with ethylene glycol (50 ml) containing KOH (9·0 g) for 3 hr. The amine evolved was trapped in saturated aqueous picric acid, which afforded trimethylamine picrate, m.p. and mixed m.p. 216 °C.

The reaction mixture was separated in the usual manner into dihydromethine-II (1·4 g) and dihydromethine-I (4·5 g) which was recycled to yield more dihydromethine-II. This methine, an oil, could not be separated into its constituents by chromatography.

(v) A solution of dihydromethine-II (3·3 g) in dry benzene (25 ml) and dry pyridine (2·0 ml) was kept below 20 °C whilst osmium tetroxide (3·0 g) was added. After 20 hr at room temperature the dark mixture was diluted with benzene (100 ml) and shaken with 1% KOH containing 10% mannitol (5 × 50 ml). The benzene solution after washing and drying yielded a pale brown gum which did not crystallize.

The gum was dissolved in ethanol (100 ml) and the solution treated at room temperature with potassium periodate (2·0 g) in dilute H_2SO_4 (100 ml of 1·5N). After keeping for 6 hr,

the reaction mixture was diluted and exhausted with ether. Removal of the ether left an oil which was diluted with water (100 ml) and distilled into DNP reagent.

The precipitate, which was collected by benzene, yielded an orange-yellow solid (2·3 g). Paper chromatography according to the method of Huelin (1952) showed it to be a mixture of the DNP's of pentanal and hexanal. Some difficulty was experienced in isolating these substances. The mixture was first chromatographed on acid-washed alumina in benzene–light petroleum (30 : 70), the appropriate eluates, as judged by the intensities of the spots of paper chromatograms, combined and rechromatographed on bentonite-kieselguhr (Elvidge and Whalley 1955) using benzene–carbon tetrachloride (90 : 10). Eventually, after repeated chromatography and recrystallization from ethanol, pentanal DNP, m.p. and mixed m.p. 102–103 °C (Found : C, 50·3 ; H, 5·4%. Calc. for $C_{11}H_{14}O_4N_4$: C, 49·6 ; H, 5·2%) and hexanal DNP, m.p. and mixed m.p. 105–106 °C (Found : C, 51·5 ; H, 6·0%. Calc. for $C_{12}H_{16}O_4N_4$: C, 51·4 ; H, 5·8%), were isolated.

The non-volatile material from the oxidation was a viscous, pale brown oil which did not crystallize. A small amount of it was converted to the DNP, which after chromatography in benzene on acid-washed alumina and several recrystallizations from ethyl acetate afforded glistening orange *needles*, m.p. 166–167 °C (Found : C, 60·8 ; H, 6·9%. Calc. for $C_{21}H_{26}O_5N_4$: C, 60·6 ; H, 6·8%).

(vi) A solution of the non-volatile aldehyde (3·0 g) and silver nitrate (12·0 g) in ethanol (30 ml) and water (15 ml) was treated with NaOH (4·5 g) in water (10 ml) and ethanol (10 ml), and the mixture shaken for 20 hr. The brown precipitate was collected and washed with water and ethanol. The combined filtrate and washings were acidified and extracted with ether (5 × 50 ml). The acidic material was removed from the ether with alkali and transferred back to ether after acidification. Removal of the ether left an amber gum (2·6 g) which was fractionally crystallized from ethyl acetate, to yield crude acid " A ", m.p. 141–147 °C (1·0 g) and crude acid " B ", m.p. 130–137 °C (0·6 g).

The combined filtrates from the fractionation were evaporated and the residue after methylation with diazomethane, chromatographed on acid-washed alumina. The light petroleum eluate contained a colourless oil which after saponification yielded a further amount (0·3 g) of acid " B ".

Recrystallization of crude " A " from ethyl acetate gave colourless *prisms* (0·9 g), m.p. 156–158 °C, $[\alpha]_D^{14}$ +33° (*c*, 0·78) (Found : C, 72·3 ; H, 9·7%. Calc. for $C_{16}H_{26}O_3$: C, 72·1 ; H, 9·8%).

Crude acid " B " on recrystallization from a little ethyl acetate afforded large colourless *needles* (0·85 g), m.p. 144 °C, $[\alpha]_D^{14}$ +19° (*c*, 0·75) (Found : C, 71·4 ; H, 9·5 ; (*C*)CH_3, 5·0%. Calc. for $C_{15}H_{24}O_3$: C, 71·4 ; H, 9·6 ; 1 × (*C*)CH_3, 6·0%).

(*j*) *Barbier-Wieland Degradation of the C_{16} Acid.*—(i) The acid (0·69 g) was converted to the oily methyl ester in the usual way with diazomethane. The ester in benzene (10 ml) was added slowly to the Grignard reagent from bromobenzene (4·5 g), magnesium (0·7 g), and ether (20 ml). After refluxing for 4 hr the reaction mixture was worked up as usual to yield the *carbinol* (63% yield) which crystallized from ethanol in colourless rectangular plates, m.p. 183–184 °C (Found : C, 83·5 ; H, 8·9%. Calc. for $C_{28}H_{36}O_2$: C, 83·1 ; H, 9·0%).

(ii) The carbinol (0·4 g) was dehydrated by refluxing in glacial acetic acid (10 ml) containing H_2SO_4 (0·1 ml) for 8 hr. The product, a brown oil, was ozonized in acetic acid (15 ml) at room temperature for 0·5 hr. Zinc dust was added to reduce the ozonide, the mixture diluted with water, and the product (0·2 g), a brown gum, recovered with ether.

Without removing the benzophenone, the gum was oxidized with silver nitrate (3 g), NaOH (1·5 g) in water (15 ml), and ethanol (15 ml) as in (*i*) (vi) above. The acidic product was converted to its methyl ester, which after purification by chromatography, was saponified. The acid crystallized from a little ethyl acetate in colourless needles, m.p. 144 °C, undepressed by admixture with the C_{15} acid.

(*k*) *Barbier-Wieland Degradation of the C_{15} Acid.*—(i) The methyl ester prepared from the acid (0·9 g) was reacted with the Grignard reagent prepared from bromobenzene (9·5 g) as in (*j*) (i) above. The only product was steam distilled to remove diphenyl and then chromatographed in light petroleum on alumina (20 g). The benzene–light petroleum (50 : 50) eluate yielded a

gum which crystallized from hexane in colourless *needles* (0·9 g), m.p. 141–142 °C (Found : C, 80·7 ; H, 9·2%. Calc. for $C_{21}H_{28}O_2$: C, 80·7 ; H, 9·0%).

(ii) To the phenyl ketone (0·8 g) in dry ether (20 ml) was added ethereal phenyl-lithium (10 ml, containing 1·05 g) in an atmosphere of nitrogen. After refluxing for 4 hr, the mixture was kept overnight, then water was added, and the product isolated as usual. It was an amber gum which yielded no ketone on chromatography but which could not be crystallized.

The carbinol was dehydrated by refluxing it with acetic acid (10 ml) and acetic anhydride (5 ml) for 7 hr. The solution was diluted, basified with ammonia, and extracted with chloroform. Evaporation of the solvent gave a dark brown gum which was purified by chromatography to yield an amber gum which did not crystallize.

The diphenylethylene (0·6 g) was degraded by ozonolysis and oxidation with silver oxide as in (*i*). The crude acidic fraction (0·1 g) crystallized on addition of a few drops of acetone. It crystallized from acetone in *prisms*, m.p. 209 °C, $[\alpha]_D^{14}$ +5·5° (*c*, 0·91) (Found : C, 70·4 ; H, 9·3 ; (*C*)CH_3, 5·1%. Calc. for $C_{14}H_{22}O_2$: C, 70·6 ; H, 9·3 ; 1×(*C*)CH_3, 6·3%).

(*l*) *Exhaustive Methylation of the Anhydrodiol.*—(i) The methiodide (11·7 g) was decomposed in an atmosphere of nitrogen as in (*i*). The basic fraction, an oil (8·1 g), was chromatographed on alumina (100 g), light petroleum, benzene, ether, and ethanol eluates being collected.

The light petroleum eluate contained the isoanhydrodiol, an oil, which yielded a *methiodide* crystallizing from ethanol–ethyl acetate in colourless needles, m.p. 167–168 °C, $[\alpha]_D^{14}$ +32° (*c*, 0·63) (Found : C, 58·4 ; H, 8·6%. Calc. for $C_{23}H_{40}ONI$: C, 58·3 ; H, 8·5%).

The benzene eluate afforded a pale brown oil, methine-I, which was purified by further chromatography. It formed a *methiodide*, colourless needles, m.p. 172–175 °C (Found : C, 58·9 ; H, 8·6%. Calc. for $C_{24}H_{42}ONI$: C, 59·1 ; H, 8·7%).

The ether eluates contained isomethine-I, an oil, which yielded a hygroscopic *methiodide* colourless plates from ethyl acetate, m.p. 194 °C (Found : C, 58·6 ; H, 8·6%. Calc. for $C_{24}H_{42}ONI$: C, 59·1 ; H, 8·7%).

(ii) A solution of the isomethine-I (1·7 g) in acetone (25 ml) was shaken and treated gradually with powdered $KMnO_4$ until it was no longer decolorized. The brown precipitate was collected, washed with ether, suspended in dilute acid, and treated with sodium bisulphite. The liberated acid, isolated with the aid of ether, crystallized from acetone in prisms, m.p. and mixed m.p. with the C_{14} acid of (*k*) (ii), 209 °C.

The combined acetone filtrate and ether washings from the oxidation were evaporated and the neutral fraction isolated from the residue. It was a colourless gum which slowly crystallized on keeping, but recrystallization was unsatisfactory. Ready purification, however, was achieved by sublimation at 80–90 °C/1 mm. The *product* formed colourless plates, m.p. 96–97 °C, or prisms, m.p. 96–97 °C, on recrystallization from hexane, and had $[\alpha]_D^{22}$ +87° (*c*, 1·22) (Found : C, 74·9 ; H, 9·8%. Calc. for $C_{13}H_{20}O_2$: C, 74·9 ; H, 9·7%). The ketone gave a negative Zimmermann test for an active methylene group. It formed a *DNP* which crystallized from ethanol in orange-red prisms, m.p. 144–145 °C (Found : C, 58·7 ; H, 6·3%. Calc. for $C_{19}H_{24}O_5N_4$: C, 58·8 ; H, 6·2%).

(iii) Catalytic hydrogenation of methine-I (1·0 g) in ethanol (20 ml) in the presence of platinum oxide was very slow after the uptake of 1 mol. of hydrogen. The product was a gum which afforded *dihydromethine-I methiodide*, colourless needles from ethanol–ethyl acetate, m.p. 175–176 °C, $[\alpha]_D^{14}$ +37° (*c*, 0·81) (Found : C, 58·8 ; H, 9·1%. Calc. for $C_{23}H_{42}ONI$: C, 58·9 ; H, 9·1%).

(iv) The above methiodide (4·9 g) was decomposed with ethylene glycolic KOH as usual, when trimethylamine was evolved. The mixture was diluted and extracted with ether (4×50 ml). On shaking the extract with 5% HCl, the sparingly soluble *dihydromethine-I hydrochloride* separated as an oil which solidified on keeping. It crystallized from ethyl acetate in colourless needles, m.p. 165–166 °C (Found : C, 71·3 ; H, 11·2%. Calc. for $C_{23}H_{42}ONCl$: C, 71·9 ; H, 11·0%). *Dihydromethine-I*, regenerated from this substance, then crystallized from hexane at 0 °C in large colourless needles, m.p. 62–63 °C, $[\alpha]_D^{14}$ +5·2° (*c*, 9·97) (Found : C, 79·3 ; H, 11·8%. Calc. for $C_{23}H_{41}ON$: C, 79·5 ; H, 11·9%).

Evaporation of the ether solution gave dihydromethine-II (1·7 g) as a gum. Additional amounts were obtained by recycling recovered dihydromethine-I, the final yield being 2·8 g. The gum was ozonized in acetic acid (10 ml) at 10 °C for 1·5 hr. The ozonide was decomposed by zinc dust, water (30 ml) added, and the mixture partially distilled into DNP reagent. Pentanal and hexanal DNP's were isolated and identified as in (*i*) (v).

The non-volatile fraction was isolated as a brown viscous oil which was chromatographed on acid-washed alumina (20 g). The light petroleum eluate was a brown gum (0·6 g). A portion of it was converted to its DNP, which after elution from acid-washed alumina with ether, afforded orange needles from ethyl acetate–ethanol, m.p. 219–220 °C, of the *DNP of the* αβ-*unsaturated* aldehyde (Found : C, 61·6 ; H, 6·8%. Calc. for $C_{22}H_{26}O_5N_4$: C, 61·7 ; H, 6·6%).

The benzene eluate also contained a gum (0·3 g) which yielded the same DNP, but the ether eluate (0·5 g) did not.

(v) The material eluted by light petroleum was oxidized in acetone (10 ml) with $KMnO_4$, and the product isolated as in (*l*) (ii). The C_{13} ketone (0·02 g) and the C_{14} acid (0·05 g) were obtained.

The benzene eluate on oxidation gave the C_{13} ketone (0·02 g) and the C_{14} acid (0·05 g), and the ether eluate also gave similar yields of these two substances.

(*m*) *Anhydrodiol N-Oxide.*—A solution of perphthalic acid (4·0 g) in ether (180 ml) was added to a solution of the anhydrodiol (2 g) in ether (50 ml) and chloroform (50 ml) at 5 °C. After keeping at room temperature for 60 hr, most of the solvent was removed, chloroform was added, and the solution washed in turn with aqueous sodium carbonate, aqueous sodium thiosulphate, and water. Evaporation of the solvent left a semicrystalline mass which crystallized from ethanol–acetone in colourless *prisms* (1·15 g), m.p. between 185 and 195 °C (decomp.), depending on the rate of heating, $[\alpha]_D^{14}$ +10·5° (*c*, 1·15) (Found : C, 75·9 ; H, 10·6%. Calc. for $C_{22}H_{37}O_2N$: C, 76·0 ; H, 10·7%).

Reduction of the substance (0·2 g) in 5% HCl (10 ml) and ethanol (10 ml) with zinc dust on the steam-bath for 2 hr regenerated the anhydrodiol (0·14 g).

(*n*) *Permanganate Oxidation of the Anhydrodiol.*—Powdered $KMnO_4$ (7 g) was added in small amounts, with occasional shaking, over a period of 6 days to a solution of the anhydrodiol (2 g) in acetone (60 ml) at 0–5 °C. The brown precipitate was collected, washed well with ether, and then suspended in dilute acid. Powdered sodium bisulphite was added carefully until all the MnO_2 had been reduced. The liberated acid, isolated with the aid of ether, crystallized from acetone–light petroleum in colourless plates (0·38 g), m.p. and mixed m.p. with the C_{14} acid, 209 °C. By saponifying the benzene eluate from a chromatogram of the methyl esters of the acidic material in the mother liquors, an additional 0·05 g crystalline acid was obtained.

The combined acetone–ether solutions yielded a neutral oil (0·14 g) which on sublimation at 90–100 °C/1 mm yielded the C_{13} ketone (0·05 g), m.p. and mixed m.p. 96–97 °C.

(*o*) *Permanganate Oxidation of Himbacine.*—Himbacine (4 g) in acetone (80 ml) was oxidized with $KMnO_4$ (14 g) at 0–5 °C during 5 days, and the products isolated as in (*n*).

The acidic fraction was a gum (2 g) which deposited crystals on addition of a little ethyl acetate. Recrystallization from ethyl acetate gave the *lactone acid* (0·55 g) as large colourless prisms, m.p. 194–196 °C, $[\alpha]_D^{23}$ +35° (*c*, 0·89) (Found : C, 66·5 ; H, 8·0%. Calc. for $C_{14}H_{20}O_4$: C, 66·6 ; H, 8·0%). A further amount of the acid (0·51 g) was isolated from the mother liquors by a procedure similar to that described in (*n*).

The neutral material in the acetone–ether solution was a brown viscous oil (0·32 g). On sublimation at 110 °C/1 mm, it yielded the *keto-lactone* (0·065 g), fine colourless needles, m.p. 112 °C, $[\alpha]_D^{22}$ +110° (*c*, 1·05) (Found : C, 70·0 ; H, 7·8%. Calc. for $C_{15}H_{18}O_3$: C, 70·2 ; H, 8·2%). It gave negative tests with ferric chloride and with tetranitromethane.

The *DNP* crystallized from benzene–light petroleum in golden-yellow prisms, m.p. 242 °C (decomp.) (Found : C, 56·8 ; H, 5·6%. Calc. for $C_{19}H_{22}O_6N_4$: C, 56·7 ; H, 5·5%).

Himbacine (0·21 g) was recovered from the acetone–ether solution prior to the isolation of the neutral fraction.

(*p*) *Chromic Acid Oxidation of Himbacine.*—A solution of himbacine (1·0 g) in glacial acetic acid (15 ml) was treated dropwise with a solution of chromic anhydride (1·5 g) in glacial acetic acid (10 ml) and water (1 ml) with stirring at 70 °C. The reddish brown oil which separated dissolved as the oxidation proceeded. After 4 hr, the reaction mixture was cooled, diluted, and extracted with ether (5 × 50 ml). The extract was washed with water and then with saturated sodium bicarbonate. The bicarbonate washings were acidified and extracted with ether. From this extract only a little acetic acid and a trace of other acidic material could be isolated.

The ether solution containing the neutral fraction, on evaporation gave a crystalline residue (0·06 g) which on sublimation at 100–110 °C/1 mm yielded the keto-lactone, m.p. and mixed m.p. 112 °C.

The green acidic solution on working up in the usual manner afforded unchanged himbacine (0·35 g).

Oxidation at lower or higher temperatures did not improve the yield of the keto-lactone.

(*q*) *Chromic Acid Oxidation of the Anhydrodiol.*—Oxidation of the anhydrodiol (0·6 g) with chromic anhydride (1·2 g) was effected at 70 °C and the products isolated as in (*p*).

The neutral fraction (0·048 g) on sublimation gave the keto-lactone, m.p. and mixed m.p. 11– °C.

The acidic fraction was a brown gum (0·05 g) from which crystalline products could not be isolated.

The basic fraction (0·35 g) crystallized from light petroleum in colourless needles, m.p. and mixed m.p. with himbacine, 132 °C.

(*r*) *Oxidation of the* $C_{14}H_{22}O_3$ *Acid.*—A solution of the acid (0·1 g) $KMnO_4$ (0·1 g) and KOH (0·1 g) in water (7 ml) was heated on the steam-bath for 0·5 hr, by which time all the permanganate had been consumed. MnO_2 was reduced by the addition of sodium bisulphite and dilute acid and the mixture shaken with ether. On working up in the usual manner, the lactone acid (0·045 g), m.p. and mixed m.p. 196 °C, was obtained.

(*s*) *Conversion of the Lactone Acid to the Keto-lactone.*—(i) The crude acid chloride, prepared from the lactone acid (0·6 g) in the usual way using thionyl chloride, was refluxed in dioxan solution (10 ml) with sodium borohydride (0·1 g) for 1·5 hr. The mixture was cooled, diluted, and extracted with ether. The neutral fraction, isolated in the usual manner, on crystallization from ether–light petroleum, afforded the *primary alcohol* (0·45 g) as colourless needles, m.p. 166 °C (Found : C, 70·2 ; H, 9·1%. Calc. for $C_{14}H_{22}O_3$: C, 70·6 ; H, 9·3%).

(ii) The alcohol (0·4 g) was converted to its methanesulphonate by treatment with methanesulphonyl chloride in pyridine at room temperature for 48 hr. The crude compound was refluxed in collidine (8 ml) for 1·5 hr. Working up in the usual manner gave a crude product which was purified by sublimation ; the fraction collected in the range 90–140 °C/0·5 mm was resublimed at 100–110 °C/0·5 mm to yield the *ene-lactone* (0·07 g) which crystallized from pentane in needles, m.p. 93–96 °C (Found : C, 75·6 ; H, 9·1%. Calc. for $C_{14}H_{20}O_2$: C, 76·4 ; H, 9·1%) (bands in the i.r. (carbon disulphide) at 1640 and 895 cm^{-1}).

(iii) A mixture of the ene-lactone (0·043 g) and osmium tetroxide (0·07 g) in dioxan (1 ml) was allowed to stand in the dark for 7 days. The precipitated osmate was decomposed with hydrogen sulphide to yield a gum (0·03 g), which had i.r. absorption at 3500 cm^{-1} (hydroxyl) but no strong absorption in the 1600 and 900 cm^{-1} regions, consonant with a glycol structure.

The crude diol in methanol (5 ml) was treated with sodium metaperiodate (0·05 g) in water (4 ml). After 24 hr, water (10 ml) was added and the product isolated by the aid of ether. Sublimation at 100–110 °C/0·5 mm gave colourless needles (0·008 g) of the keto-lactone, identified by m.p. and mixed m.p. and i.r. spectrum.

(*t*) *Dehydrogenation of the Lactone Acid.*—A mixture of the acid (1·6 g) and 10% palladium–charcoal (2·0 g) was heated at 320 °C in an atmosphere of nitrogen for 3 hr. The residue was extracted thoroughly with ether, the solution washed in turn with saturated sodium bicarbonate, 5% NaOH and water, and dried. Evaporation gave a colourless oil (0·35 g) which was dissolved in hot ethanol (5 ml) and treated with TNB (0·3 g). On cooling, a pale yellow adduct separated in fine needles (0·34 g), m.p. 86–87 °C, undepressed by admixture with an authentic specimen of

the *TNB adduct of 2-ethylnaphthalene* (m.p. 88 °C) (Found: C, 58·4; H, 4·3%. Calc. for $C_{18}H_{15}O_6N_3$: C, 58·5; H, 4·1%). As this adduct tended to lose hydrocarbon under reduced pressure, the analytical sample was dried at room temperature and pressure.

The sodium bicarbonate extract on acidification yielded a gum (0·21 g) from which no crystalline substance could be isolated. The NaOH extract contained only traces of material.

(*u*) *Dehydrogenation of the* $C_{14}H_{22}O_3$ *Acid.*—The acid (1·4 g) was dehydrogenated as in (*t*) and the residue extracted with chloroform. After washing with aqueous sodium bicarbonate and NaOH, the chloroform was removed and the residue chromatographed in light petroleum (b.p. 40 °C) on alumina. The light petroleum eluate yielded a colourless oil which afforded an adduct with TNB crystallizing from ethanol in lemon yellow needles (0·41 g), m.p. 130 °C (Found: C, 59·4; H, 4·6%. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·5; H, 4·4%). It was identified as the TNB adduct of 2-ethyl-3-methylnaphthalene as described in Section III.

Only traces of material were present in the alkaline extracts.

(*v*) *Reduction Products of the* $C_{14}H_{22}O_3$ *Acid and their Dehydrogenation.*—(i) The oily methyl ester prepared from the acid (0·91 g) by diazomethane, was dissolved in dry ether (30 ml), and added slowly to lithium aluminium hydride (0·2 g) in ether (20 ml). After keeping at room temperature for 1½ hr, water and then dilute acid were added, and the mixture worked up as usual. The *alcohol* crystallized from light petroleum in large colourless prisms (0·83 g), m.p. 107–108 °C, $[\alpha]_D^{14}$ +10·5° (*c*, 0·98) (Found: C, 75·0; H, 10·9%. Calc. for $C_{14}H_{24}O_2$: C, 75·0; H, 10·8%).

Dehydrogenation of the alcohol (0·75 g) as in (*t*) gave an oil (0·3 g), from which a TNB adduct was obtained as yellow needles from ethanol, m.p. 91–93 °C (Found: C, 59·5; H, 4·6%. Calc. for $C_{20}H_{19}O_6N_3$: C, 60·5; H, 4·8%. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·5; H, 4·4%). The product was obviously a mixture but no separation could be achieved by repeated recrystallizations.

(ii) The *p-toluenesulphonate*, prepared in dry pyridine as usual, crystallized from aqueous methanol in colourless plates, m.p. 102–103 °C (Found: C, 66·7; H, 8·1%. Calc. for $C_{21}H_{30}O_4S$: C, 66·6; H, 8·0%).

(iii) The reduction of the tosylate (0·41 g) by lithium aluminium hydride (0·06 g) in ether (15 ml) as usual gave a crude product which was purified by chromatography to yield the deoxy derivative as a colourless oil (0·2 g), which did not crystallize.

(iv) Heating the deoxy derivative (0·5 g) with sulphur (0·23 g) at 210–230 °C for 4·5 hr gave a gum from which a crystalline product or TNB adduct could not be obtained.

The deoxy derivative (0·55 g) was dehydrogenated by heating with palladium–charcoal (0·8 g) at 320 °C and the mixture worked up as usual. The adduct with TNB (yield 0·21 g) crystallized from ethanol in yellow needles, m.p. 93–97 °C, which four recrystallizations raised to 102–104 °C, but the product was clearly a mixture (Found: C, 60·1; H, 4·9%. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·5; H, 4·4%. Calc. for $C_{20}H_{19}O_6N_3$: C, 60·5; H, 4·8%). Dehydrogenation at 280–300 °C also gave a mixture.

(*w*) *Reduction of the Lactone Acid.*—The methyl ester prepared from the lactone acid (0·4 g), was reduced with lithium aluminium hydride (0·3 g) in ether (50 ml) at room temperature for 15 hr. The product (0·35 g) obtained in the usual manner was a gum, which was heated on the steam-bath for 3 hr with a 5% solution of H_2SO_4 in water–dioxan (70 : 30) (30 ml). After dilution, the mixture was worked up as usual to yield the "*isomeric alcohol*" which crystallized from light petroleum in colourless plates (0·27 g), m.p. 125–126 °C, $[\alpha]_D^{14}$ +51° (*c*, 1·25) (Found: C, 74·8; H, 10·9%. Calc. for $C_{14}H_{24}O_2$: C, 75·0; H, 10·8%). The i.r. spectrum (Nujol) had absorption at 3430 cm^{-1} (hydroxyl).

(*x*) *Degradation of the Keto-lactone.*—The keto-lactone (0·45 g) was shaken with 2% aqueous KOH (20 ml) at room temperature until it dissolved. The solution was washed with ether, acidified with 5% HCl, and immediately extracted with ether. The ether extract on evaporation gave an uncrystallizable gum (0·42 g) which was dissolved in methylene chloride (20 ml) and ozonized at −20 °C for 1 hr. Water (20 ml) was added and the methylene chloride and water (16 ml) distilled into DNP reagent.

After shaking the mixture for 1 hr, the organic layer was separated and the solution extracted again with methylene chloride. The combined extracts on working up as usual gave a yellow-orange crystalline residue which was dissolved in benzene and passed through a short column of acid-washed alumina. The benzene was removed and the residue crystallized from ethanol to yield yellow-orange plates (yield 48%), m.p. and mixed m.p. with authentic acetaldehyde DNP, 162 °C. The identification was confirmed by paper chromatography (Huelin 1952).

The undistilled aqueous solution was basified with 5% KOH (1 ml) and 30% H_2O_2 (2 ml) added. After 1 hr the solution was acidified and a little MnO_2 added to decompose excess H_2O_2. The solution was saturated with salt and extracted repeatedly with ether. The extract gave an amber gum (0·35 g) which when heated at 120–150 °C evolved CO_2, leaving a brown residue. This was dissolved in benzene and chromatographed on silica gel (15 g), 15 ml fractions being collected. The benzene–ether (80 : 20) eluate (fractions 7–12) yielded a colourless gum (0·1 g) which crystallized on addition of benzene. The solid was collected, washed with benzene, and recrystallized from water to yield colourless *prisms*, m.p. 170 °C, $[\alpha]_D^{15}$ +47° (*c*, 0·82 in ethanol) (Found : C, 60·4 ; H, 8·0%. Calc. for $C_{16}H_{16}O_4$: C, 60·0 ; H, 8·1%). When mixed with racemic β-(*trans*-2-carboxycyclohexyl)propionic acid of m.p. 140 °C, the m.p. was 145–155 °C. The i.r. spectra (Nujol) of the two substances were identical.

(*y*) *Synthesis of* β-(trans-*2-Carboxycyclohexyl*)*propionic Acid.*—(i) Crude 3,4-dihydro-2-hydroxymethylene-1(2*H*)-naphthalenone (5·5 g) (Campbell, Campbell, and Schrage 1950) was dissolved in 4% NaOH (50 ml), 30% H_2O_2 added with cooling, and the mixture kept at room temperature for 3 hr. The dark solution was acidified and extracted with ether, from which the acidic product was recovered by extraction with saturated sodium bicarbonate After acidification, the crude product was isolated with ether as usual and formed a reddish brown crystalline solid. By washing with a little chloroform and recrystallizing from water, β-(*o*-carboxyphenyl)-propionic acid was obtained as colourless prisms, m.p. 164 °C (lit. 166 °C).

(ii) Catalytic hydrogenation of the above acid (0·4 g) in glacial acetic acid (20 ml) using platinum oxide (0·4 g) at room temperature and pressure was complete in 3 hr. The crude β-(*cis*-2-carboxycyclohexyl)propionic acid obtained by filtration and evaporation of the solvent had m.p. 95–100 °C (lit. 103 °C) and was used directly in the next step.

(iii) The *cis*-acid was isomerized to the *trans*-acid by HCl at 180 °C by the method of Hückel, Reverey, and Windaus (1925). It had m.p. 140 °C (lit. 143 °C).

(*z*) *Synthesis of 1,3-Dimethyl-2-ethylnaphthalene.*—(i) A cooled solution of the sodio derivative of ethyl α-propionylpropionate, obtained by heating the ester (15 g) with a solution of sodium (2·17 g) in methanol (25 ml) and benzene (25 ml), was treated with a solution of the methiodide of 2-diethylaminocyclohexanone (17·3 g) in methanol (25 ml). The solution was kept overnight, and then refluxed for 3 hr. Water (100 ml) was added and the product, a brown oil, isolated with ether as usual.

The residual alkaline solution was acidified, when a crystalline *acid* separated. It was worked up as usual and recrystallized from ethyl acetate as colourless needles (1·2 g), m.p. approx. 129 °C (decarboxylation ; m.p. depends on rate of heating) (Found : C, 70·3 ; H, 8·1%. Calc. for $C_{13}H_{16}O_2$: C, 70·2 ; H, 8·2%). It was assigned the structure (XXVIII) as discussed in Section XII.

The neutral product was refluxed under nitrogen for 3 hr with methanol (25 ml) and 50% KOH (3 ml). The solution was concentrated to half its volume, diluted with water, and extracted with ether. The ketone (XXIX), a colourless oil (6·0 g), isolated by distillation, had b.p. 122–126 °C/1–2 mm. Its *DNP derivative* crystallized from ethyl acetate in crimson plates, m.p. 178 °C (Found : C, 60·4 ; H, 6·2%. Calc. for $C_{18}H_{22}O_4N_4$: C, 60·3 ; H, 6·2%).

(ii) The unsaturated ketone (6 g) in ethanol (20 ml) was hydrogenated over 10% palladium–charcoal (0·3 g). The product (XXX) was a colourless oil, b.p. 110–114 °C/1–2 mm. Its *DNP derivative* separated from ethyl acetate–ethanol as orange plates, m.p. 189–190 °C (Found : C, 60·0 ; H, 6·7%. Calc. for $C_{18}H_{24}O_4N_4$: C, 60·0 ; H, 6·7%).

(iii) A solution of the saturated ketone (4·5 g) in dry ether (20 ml) was added slowly to the Grignard reagent prepared from ethyl bromide (5·6 g) and magnesium (1·2 g) in dry ether (30 ml).

After refluxing the mixture for 2 hr, the crude tertiary alcohol (5·2 g) was isolated in the usual manner.

A portion of the product (1·5 g) was heated with 10% palladium–charcoal (1·0 g) under nitrogen at 280–300 °C for 3 hr. An ether extract of the residue gave a gum, a solution of which in hexane was passed through a column of alumina. The colourless oil (XXXI) (1·0 g) so obtained gave an *adduct* with TNB which crystallized from ethanol in fine yellow needles, m.p. 114–115 °C (Found: C, 60·6; H, 4·8%. Calc. for $C_{20}H_{19}O_6N_3$: C, 60·5; H, 4·8%).

(*aa*) *TNB Adducts of Known Naphthalenes.*—(i) The *adduct* of 2-isopropylnaphthalene formed pale yellow needles, m.p. 106 °C, from ethanol (Found: C, 59·4; H, 4·5%. Calc. for $C_{19}H_{17}O_6N_3$: C, 59·5; H, 4·4%).

(ii) 2,5-Dimethyl-3-ethylnaphthalene, regenerated from the picrate by passing its hot benzene solution through alumina, gave a *TNB adduct*, lemon-yellow needles from ethanol, m.p. 127–128 °C (Found: C, 60·6; H, 5·0%. Calc. for $C_{20}H_{19}O_6N_3$: C, 60·5; H, 4·8%).

Himbeline

(*a*) *Himbeline and Derivatives.*—The alkaloid crystallized from hexane in colourless needles, m.p. 100 °C (Found: C, 76·3; H, 10·1; (*N*)CH_3, nil; (*C*)CH_3, 5·8%. Calc. for $C_{21}H_{33}O_2N$: C, 76·1; H, 10·0, 1×(*C*)CH_3, 4·5%. Calc. for $C_{22}H_{35}O_2N$: C, 76·5; H, 10·2; 1×(*C*)CH_3, 4·4%).

The *acetyl derivative* formed colourless needles from light petroleum, m.p. 159 °C (Found: C, 74·3; H, 9·4; N, 3·5%. Calc. for $C_{23}H_{35}O_3N$: C, 74·0; H, 9·4; N, 3·8%).

The *methanesulphonyl derivative* crystallized from acetone in colourless needles, m.p. 155 °C (Found: C, 64·6; H, 8·7%. Calc. for $C_{22}H_{35}O_4NS$: C, 64·5; H, 8·9%). Both substances were recovered unchanged after keeping with 5% methanolic KOH at room temperature for 100 hr.

(*b*) *Methylation to Himbacine.*—The base (0·5 g), 98% formic acid (0·3 g), and 35% formaldehyde (0·3 g) were heated on the steam-bath for 2 hr. By working up in the usual manner, himbacine (0·32 g), m.p. and mixed m.p. 132 °C, was obtained. The identity of the samples was checked by their i.r. spectra.

(*c*) *Dihydrohimbeline.*—The alkaloid (0·6 g) in glacial acetic acid (20 ml) was hydrogenated over platinum oxide (0·05 g) at atmospheric pressure. The product, isolated by the usual procedure, was a gum, which on treatment with ethanolic HCl afforded a *hydrochloride*, colourless needles from ethanol–ether, m.p. 285 °C, $[\alpha]_D^{14}$ +55° (*c*, 0·84 in 95% ethanol) (Found: C, 67·9; H, 9·9%. Calc. for $C_{21}H_{36}O_2NCl$: C, 68·2; H, 9·8%).

Dehydrogenation of the base (1·0 g) with palladium–charcoal as in (*g*) gave dehydrohimbacine (0·3 g).

(*d*) *Permanganate Oxidation of Himbeline.*—Oxidation of himbeline (1·0 g) with $KMnO_4$ (4 g) was carried out as in (*o*). The lactone acid (0·38 g, 50% yield) and the keto-lactone (0·02 g) were isolated.

(*e*) *Action of Performic Acid.*—A solution of himbeline (0·6 g) in 98% formic acid (6 g) and 30% H_2O_2 (0·6 g) was heated at 40 °C for 10 hr and then kept at room temperature for 60 hr. The solution was concentrated under reduced pressure, the residual gum dissolved in water, and the solution basified with 5% NaOH, when a precipitate formed. The product, *himbeline epoxide*, isolated by ether extraction, crystallized from benzene–light petroleum in colourless prisms (0·2 g), m.p. 192–193 °C (Found: C, 72·8; H, 9·1%. Calc. for $C_{21}H_{33}O_3N$: C, 72·6; H, 9·6%). The i.r. spectrum (Nujol) had bands at 3310 cm^{-1} (imino group), 1770 cm^{-1} (γ-lactone), and strong bands between 900 and 800 cm^{-1} (epoxide), but no band at 985 cm^{-1} (*trans*-double bond).

On acidifying the alkaline solution and then basifying carefully with ammonia, a crystalline precipitate formed. The substance, *himbeline glycol*, separated from acetone–light petroleum in colourless laths (0·1 g), m.p. 196–198 °C (strongly depressed on admixture with the first product) (Found: C, 68·9; H, 9·5%. Calc. for $C_{21}H_{35}O_4N$: C, 69·0; H, 9·7%). The i.r. spectrum showed bands at 3470 cm^{-1} (hydroxyl), 3300 cm^{-1} (imino group), and 1745 cm^{-1} (hydrogen-bonded γ-lactone).

Himandravine

(*f*) *Methylation.*—Methylation, effected as in (*b*), gave an almost quantitative yield of *N-methylhimandravine*, colourless needles from hexane, m.p. 128–129 °C (strongly depressed on admixture with himbacine), $[\alpha]_D^{14}$ −7·5° (*c*, 0·94) (Found: C, 76·5; H, 10·3%. Calc. for $C_{22}H_{35}O_2N$: C, 76·5; H, 10·2%).

(*g*) *Dihydrohimandravine.*—Hydrogenation, carried out as in (*c*), gave the *product* which crystallized from hexane in fine, colourless needles, m.p. 89–90 °C, $[\alpha]_D^{14}$ +65° (*c*, 1·29) (Found: C, 75·6; H, 10·7%. Calc. for $C_{21}H_{35}O_2N$: C, 75·6; H, 10·6%).

The *hydrochloride*, prepared from ethanolic HCl, formed colourless prisms from ethanol, m.p. 309–311 °C, $[\alpha]_D^{14}$ +55° (*c*, 0·84 in 95% ethanol) (Found: C, 67·8; H, 9·6%. Calc. for $C_{21}H_{36}O_2NCl$: C, 68·2; H, 9·8%).

(*h*) *N-Methyldihydrohimandravine.*—Formaldehyde–formic acid methylation of the dihydro base, as in (*b*), yielded colourless *plates* from hexane, m.p. 98 °C, $[\alpha]_D^{14}$ +40° (*c*, 0·89) (Found: C, 76·1; H, 10·8%. Calc. for $C_{22}H_{37}O_2N$: C, 76·0; H, 10·7%).

(*i*) *Oxidation of Himandravine.*—Oxidation of the base (1·0 g) with $KMnO_4$ (3 g) by the method of (*o*) gave the lactone acid (0·39 g, 50% yield) and the keto-lactone (0·02 g).

(*j*) *Dehydrohimbacine.*—Dehydrogenation effected as in (*g*) gave dehydrohimbacine in good yield (73%).

Himgravine

(*k*) *Analyses.*—Since the alkaloid tended to assume a pink tinge it was carefully purified by chromatography and recrystallization before analysis (Found: (*C*)CH_3, 5·0; (*N*)CH_3, 4·3%. Calc. for $C_{22}H_{33}O_2N$: 1×(*C*)CH_3, 4·4; 1×(*N*)CH_3, 4·4%).

(*l*) *Catalytic Hydrogenation.*—A solution of the purified alkaloid (0·195 g) in absolute ethanol (50 ml) was shaken in an atmosphere of hydrogen at room temperature and pressure in the presence of platinum oxide (0·1 g). After about 1 hr, when approximately 1 mol. of hydrogen had been absorbed, uptake became very slow and the reaction was stopped. The product, isolated in the usual manner, crystallized from hexane in colourless needles (0·104 g), m.p. and mixed m.p. with himbacine, 132 °C. The identification was checked by the i.r. spectra of the two samples.

XIV. Acknowledgments

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CHEMICAL CONSTITUENTS OF *EVODIA MICROCOCCA*

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Summary

Leaf constituents of two varieties of *Evodia micrococca* have been investigated. Both varieties contained palmitic acid and n-hentriacontane but while *E. micrococca* F. Muell. var. *micrococca* yielded pinoresinol dimethyl ether and a trace of essential oil, *E. micrococca* var. *pubescens* Fraser & Vickery yielded (+)-sesamin and a larger quantity of a very different essential oil.

By the nitration of pinoresinol dimethyl ether, a 5,5′,6,6′-tetranitro derivative has been prepared and the structure of Freudenberg and Dietrich's (1953) lactone has been clarified. The ultraviolet absorption spectra of the four dinitroveratroles are included.

I. Introduction

Two varieties of *Evodia micrococca* are observed in the field and are distinguished as *E. micrococca* var. *pubescens* Fraser & Vickery (which retains the leaf hairs of the juvenile form in maturity) and *E. micrococca* F. Muell. var. *micrococca*, the mature leaves of which are free from hairs. A sample of each variety was examined for the presence of the chromenes characteristic of most other *Evodia* species (Kirby and Sutherland 1956) with negative results. The first smooth-leaf sample investigated was found to contain much pinoresinol dimethyl ether. Examination of additional samples, however, showed only traces of this lignan but revealed differences in composition between the two varieties which show an interesting chemical facet to the botanical variation.

II. Discussion

Single samples of the two varieties were worked up by the procedure previously employed for the isolation of chromenes from other *Evodia* species—glycerol distillation of the ether extract of the dried leaves. Both varieties yielded considerable ether extracts (8·86 and 7·96% of dry leaf weight) but minor proportions of glycerol-volatile oil (0·26 and 0·87%), consisting of neutrals (0·12 and 0·47%), phenols (0·10 and 0·13%), and acids (0·03 and 0·17%) for var. *microrocca* and var. *pubescens* respectively. The ether extract of the former deposited a considerable quantity (2·9%) of crystalline pinoresinol dimethyl ether which was filtered off before commencing the glycerol distillation. Treatment of the various fractions by extraction, chromatography, and seeding yielded no phenolic or methoxylated chromenes as was also the case with *E. bonwickii* (Kirby and Sutherland 1956). Both varieties yielded palmitic acid and a paraffin

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or mixture of paraffins (Chibnall and Piper 1934), the melting point of which agrees most closely with that of n-hentriacontane. Only trace quantities of other crystalline substances could be isolated and none of these were identified.

The well-known lignan, pinoresinol dimethyl ether (I ; $R_1=R_2=H$) is the enantiomorph of (−)-eudesmin, a constituent of the kinos of several *Eucalyptus* species (Maiden and Smith 1896 ; Robinson and Smith 1914), but was only recently first reported (Dryselius and Linberg 1956) as a plant constituent. Indeed, lignans have not previously been isolated from *Evodia* species. The identity of the isolate as pinoresinol dimethyl ether was confirmed by melting point, analysis, optical rotation, and ultraviolet spectrum and by conversion to the known 6,6′-dibromo-(I ; $R_1=Br$ and $R_2=H$) and 6,6′-dinitro-(I ; $R_1=NO_2$ and $R_2=H$) derivatives and to the diastereoisomeric epipinoresinol dimethyl ether. Pinoresinol dimethyl ether shows an *in vitro* bacteriostatic activity towards *Mycobacterium tuberculosis* (Ramaswamy and Sirsi 1957).

At this stage it appeared that var. *micrococca* leaves constituted a rich source of pinoresinol dimethyl ether whereas var. *pubescens* leaves contained little or no lignan. To provide a firmer basis for this conclusion, two further samples of each variety from a different locality were examined specifically for lignan content. Neither var. *micrococca* sample yielded crystals of pinoresinol dimethyl ether directly in the ether extract. By partitioning the ether extract between hexane and 90% methanol, saponifying the hypophase, and chromatographing the unsaponifiable fraction, small yields only (0·6 and 0·5%) of pinoresinol dimethyl ether were obtained from the var. *micrococca* samples. There was thus a surprisingly large variation (from >2·9 to 0·5%) in pinoresinol dimethyl ether content between the samples.

By the same procedure, the var. *pubescens* samples yielded by contrast, crystalline (+)-sesamin (0·3 and 0·25%), a lignan of identical structure and stereochemistry (Erdtman and Pelchowicz 1958) apart from the substitution of two methylenedioxy groups in place of the four methoxy groups of pinoresinol dimethyl ether. A trace of unidentified crystalline material isolated from the glycerol distillate from the first sample of var. *pubescens* proved to be (+)-sesamin also.

(+)-Sesamin is well known as a constituent of sesame oil, of *Asarum sieboldii* roots, and as a synergist with other insecticides (Hearon and MacGregor 1955). The identity of the isolate from *E. micrococca* var. *pubescens* was concluded from melting point, mixed melting point, analysis, optical rotation, ultraviolet spectrum, and conversion to the known dibromo derivative.

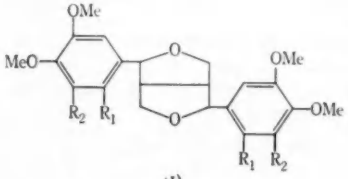
A comparison of the essential oils of the two varieties was made by submitting a combined sample of each variety to steam distillation for 6 hr and subjecting the oil obtained to gas chromatography on an Apiezon M column at 170 °C. The var. *micrococca* leaves yielded less than 0·03% of essential oil, the chromatogram of which showed 26 peaks and 3 shoulders indicating at least 29 constituents. The var. *pubescens* material yielded 0·22% of oil which showed 27 peaks and 3 shoulders. However only 11 relatively minor peaks were possibly common to both oils, which differed also in that the var. *pubescens* oil was rich in monoterpenes whereas the var. *micrococca* oil was substantially composed of

higher-boiling substances. The essential oils must thus be regarded as distinctive since they differ in yield, in general character, and in identity of most constituents.

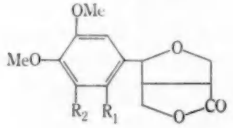
Even with the limited data presented above, it would seem very probable that the slight botanical difference between the two varieties, suggested by Mr. L. Smith (1958, personal communication) as probably the consequence of a one gene difference, is reflected in chemical differences in more than two classes of natural products. The present results require confirmation by examination of a much greater number of samples and preferably using a wider range of isolates. A similar examination of a suitable pair of Penfold's (1954) "physiological forms" (plants botanically identical but with differing essential oils) is also planned for evidence of differences concomitant with that of essential oil composition.

III. The Nitration of Pinoresinol Dimethyl Ether

According to the literature, mild nitrating conditions yield 6,6′-dinitropinoresinol dimethyl ether (Robinson and Smith 1914) and 6-nitropinoresinol dimethyl ether (Gripenberg 1946), whereas vigorous conditions result in cleavage of the veratrole-furan linkages with the formation of 4-nitro-, 4,5-dinitro- and 3,4,5-trinitroveratrole (Erdtman 1935), and bis(hydroxymethyl)succinic dilactone (Erdtman and Gripenberg 1947). The nitration conditions used by Robinson and Smith (1914) for the preparation of the dinitroveratrole yielded in our hands, however, a mixture of products separable by fractional crystallization or chromatography on alumina or by treatment with alkali, into neutral substances, mainly 4,5-dinitroveratrole, and an alkali-soluble fraction. This latter yielded a crystalline lactone, $C_{14}H_{14}N_2O_9$, m.p. 185–186 °C, presumably identical with that (m.p. 180 °C) previously obtained by Freudenberg and Dietrich (1953) by prolonged fractional crystallization of nitration products. This substance was designated as (II ; $R_1=R_2=H$) substituted by two nitro groups, the positions of which were not determined.



(I)



(II)

Since the formation of compounds such as 6-nitro-, 6,6′-dinitro-, and 6,6′-dibromopinoresinol dimethyl ether indicates position-6 is the most readily substituted, 5,6- or 2,6-disubstitution is more likely than 2,5-disubstitution. Reduction of the two nitro groups of the lactone and condensation of the resulting diamine with phenanthraquinone to yield a yellow crystalline phenanthraphenazine, confirmed the lactone as (II ; $R_1=R_2=NO_2$).

The presence of two nitro groups in the veratrole ring of this lactone suggested that a tetranitrolignan should also be produced in the nitration reaction, although no record of such compounds from this or similar lignans appears in the literature.

A search by chromatography of the residual neutral mother liquors remaining after the crystallization of the 4,5-dinitroveratrole, yielded a small quantity (1%) of the expected 5,5′,6,6′-tetranitropinoresinol dimethyl ether (I; $R_1=R_2=NO_2$) as pale yellow crystals, m.p. 221 °C. The assigned structure was confirmed by conversion to a bisphenanthraphenazine.

The nitration of pinoresinol dimethyl ether is thus analogous to the nitration of 3,4-dimethoxytoluene in yielding 6-(and 6′-)nitro and 5,6-(and 5′,6′-)dinitro substitution (Oberlin 1925; Oxford 1927) but shows in addition an alternative cleavage reaction promoted presumably by the electron-releasing properties of the furan oxygens of the lignan (de la Mare and Harvey 1957) since nitrodealkylation does not appear to have been observed in the nitration of homo-

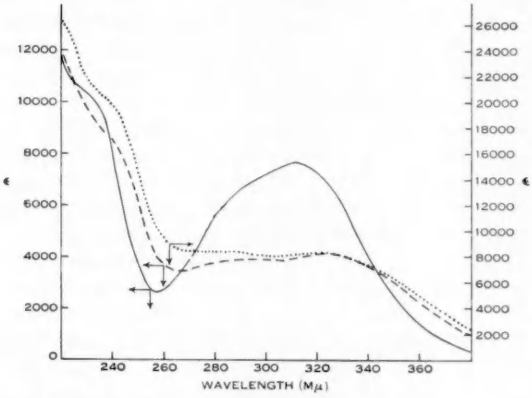


Fig. 1.—Ultraviolet absorption spectra in ethanol.
—— 3,4-Dinitroveratrole.
---- Dinitrolactone.
.... 5,5′,6,6′-Tetranitropinoresinol dimethyl ether.

veratrole or lignans lacking α-oxygenated side chains. The isolation of the dinitrolactone and the tetranitrolignan and the absence of trinitroveratrole from the nitration products formed under Robinson and Smith's conditions suggests, however, that the second (5- or 5′-) nitro group to enter the veratrole ring effectively suppresses cleavage of the veratrole-furan link. The resistance of the 5,6-dinitro compounds to cleavage at C_1 and to further nitration at C_2 was further demonstrated by refluxing the dinitro lactone in conc. nitric acid for 4 hr. The products were largely unchanged lactone and 5,6-dinitroveratric acid.

Evidence for the structures of the tetranitrolignan and the dinitrolactone was first sought by comparing their ultraviolet absorption spectra with those of the four dinitroveratroles, synthesized for this purpose. Both the lactone and tetranitrolignan can be regarded as alkylsubstituted 3,4-dinitroveratroles. Inspection of Figure 1 shows their spectra to be similar in gross features to that of 3,4-dinitroveratrole with the bathochromic shift expected from alkyl substitu-

tion. However, the extinction coefficients and wavelengths of maxima are probably influenced by steric interaction between the furan rings and the adjacent nitro group aggravating the crowding known to exist between *ortho*-nitro groups (Abe 1960). A study of molecular models supports this view and discourages attempts to draw structural conclusions from the absorption data. The spectra (see Table 1) for the 3,5-, 4,5-, and 3,6-dinitroveratroles on the other hand, afford even less satisfactory comparisons with the substances in question.

TABLE 1

ULTRAVIOLET ABSORPTION SPECTRA OF VARIOUS DINITROVERATROLES

3,4-Isomer		3,5-Isomer		3,6-Isomer		4,5-Isomer	
λ (mμ)	ε	λ (mμ)	ε	λ (mμ)	ε	λ (mμ)	ε
220	11800	220	14600	220	13500	220	10500
225	10760(s)*	245·5	8500(n)	240	5755	240·5	14100(m)
257·5	2650(n)*	255	8680(m)	245	5630(s)	265·5	6140(s)
280	5430	280	5260	250	5560	285	4450(n)
300	7180	307·5	4080(n)	287·5	1960(n)	307	4850(s)
312	7650(m)*	328	4630(m)	311	2320(m)	333	5160(m)
340	4110	340	3900	340	1610	380	2280
380	380	380	274	380	270	400	650

* (m), maximum; (n), minimum; (s), inflection point.

IV. EXPERIMENTAL

(*a*) *Extraction of* E. micrococca *var.* pubescens.—Dried leaves (SN 5558; 2·35 kg) gathered in February from Brookfield, Brisbane, on ether extraction yielded 187 g of dark oil. Working up in the same manner as in the previous paper (Kirby and Sutherland 1956) resulted in 20·4 g of glycerol-volatile oil, consisting of 11·5 g neutrals, 3·5 g phenols, and 4·0 g acids. Both the phenol fraction and the acid fraction crystallized to yield palmitic acid. The neutral fraction deposited waxy crystals which after several recrystallizations from ethyl acetate gave colourless platelets, m.p. 66·8–67·7 °C. These were optically inactive and did not dissolve in conc. H_2SO_4 (Found: C, 85·6; H, 14·9%. Calc. for $C_{31}H_{64}$: C, 85·3; H, 14·7%). Distillation of the neutrals at 0·7 mm resulted in no homogeneous fraction and the residue (1·1 g) was chromatographed on an alumina column. The first fraction yielded more crystalline paraffin. Three other crystalline substances were obtained in quantity too small for analysis, including one crystallizing from methanol as colourless needles, m.p. 121·5–122 °C. This was later shown by mixed melting point test and crystalline habit to be (+)-sesamin.

(*b*) *Extraction of* E. micrococca *var.* micrococca.—Air-dry leaves (SN 5552; 1·52 kg) gathered in January at Springbrook, Qld., were extracted with ether. The crude extract deposited crystals of pinoresinol dimethyl ether (44 g). Washing with ether and crystallization from ethanol and from ethyl acetate yielded colourless chunky crystals, m.p. 106·3–106·9 °C, $[\alpha]_D^{20}$ +63·8° (*c*, 4·6% in $CHCl_3$), light absorption in ethanol: $\lambda_{max.}$ (mμ) 232, 279; log ε 4·29, 3·76 (Found: C, 68·6; H, 6·9; OMe, 31·2%; mol. wt., 370. Calc. for $C_{22}H_{26}O_8$: C, 68·4; H, 6·8; OMe, 32·0%; mol. wt., 386). Robinson and Smith (1914) report m.p. 107 °C, $[\alpha]_D^{21}$ −64·3° for eudesmin, and Erdtman and Erdtman (1944) find $\lambda_{max.}$ 230 and 280 mμ.

The lignan was brominated (Robinson and Smith 1914) to yield the 6,6′-dibromo derivative, m.p. 171·5–172·5 °C, $[\alpha]_D^{21}$ −68·3° (*c*, 1·2% in $CHCl_3$) and nitrated to the 6,6′-dinitro derivative, m.p. 212–213 °C, $[\alpha]_D^{19}$ −122·8° (*c*, 0·8% in $CHCl_3$).

The remaining oil (91 g) yielded 4·0 g of glycerol-volatiles consisting of 1·8 g neutrals, 1·6 g phenols, and 0·5 g acids. Palmitic acid and the paraffin were isolated and identified as above. Chromatography of the neutrals on alumina yielded in addition to oils only minute amounts of the following crystalline materials in order of elution (i) paraffin, (ii) colourless crystals, m.p. 234–240 °C, (iii) colourless crystals from methanol, m.p. 75·6–77·6 °C, (iv) colourless crystals from methanol, m.p. 124–130 °C, and (v) colourless crystals from ethyl acetate, m.p. 226·5–228 °C; of these (ii) was not identical with aromadendrin and (iii) was not identical with *allo*evodione.

(*c*) *Examination of Leaf Samples for Lignan Content.*—Two leaf samples of both varieties (SN6206 and SN6207) gathered at Whian Whian, N.S.W., in June, were oven-dried and finely ground. Samples of 50 g were extracted with ether in a Soxhlet for 24 hr. The green oily residues obtained did not yield crystals directly as in (*b*) above. They were each dissolved in a mixture of 100 ml each of mutually saturated " Stanvac " hexane and 90% methanol and were equilibrated. (The distribution coefficient of pinoresinol dimethyl ether in this system is 0·06.) Each layer was then washed with 25 ml of the other solvent and the two wash layers were later equilibrated with each other. The two hypophases were combined and refluxed for 1 hr in the water-bath with the addition of KOH (5 g). After removal of the methanol, dilution with water, extraction with ether, and washing, drying, and evaporation of the ether layers, orange to red gums resulted.

The gums were dissolved in benzene and chromatographed on alumina (grade II, alkaline) columns (20 × 2·5 cm) until benzene (1 l.) had passed through. The solvent was changed to a mixture of 20% by volume of commercial chloroform in benzene. Both pinoresinol dimethyl ether and sesamin eluted after 300 ml and before 500 ml of this eluant. Rechromatography was necessary in one case where the saponification had been omitted. The crude products after one recrystallization from ethanol, melted at 102–105 °C and 102–104 °C for var. *micrococca* and 115–118 °C and 114–117 °C for var. *pubescens*. Only traces of other crystalline products were observed.

(*d*) *Identification of Sesamin.*—Mixed var. *pubescens* leaf was extracted with ether and crude sesamin, m.p. 118–121 °C isolated as in (*c*). Recrystallization from ethanol yielded laths which occasionally formed large rafts of crystals 2–3 cm long and 0·5 cm wide, of m.p. 121–122 °C, $[\alpha]_D^{22}$ +60° (*c*, 4% in $CHCl_3$), light absorption in 95% ethanol; $\lambda_{max.}$ (mμ) 235·5, 287; ε 9450, 8300 (Found: C, 67·7; H, 5·2%; OMe, nil. Calc. for $C_{20}H_{18}O_6$: C, 67·8; H, 5·1). The dibromide formed needles, m.p. 181–182 °C from ethanol. For literature values see Hearon and MacGregor (1955). There was no depression of melting point on mixing with a sample of (+)-sesamin prepared from (+)-asarinin (Davenport and Sutherland 1954).

(*e*) *Isomerization of Pinoresinol Dimethyl Ether.*—Pinoresinol dimethyl ether (7·5 g) in 675 ml ethanol and 75 ml HCl were refluxed 17 hr, diluted with 500 ml water, and then extracted with chloroform. The first crops from slow crystallization from ethanol consisted of epipinoresinol dimethyl ether, m.p. 129–130 °C, $[\alpha]_D^{29}$ +140·3° (*c*, 2·2% in $CHCl_3$) (Found: C, 68·4; H, 7·0%. Calc. for $C_{22}H_{26}O_6$: C, 68·4; H, 6·8%. The literature records m.p. 130–131 °C, $[\alpha]_D$ +141° for this substance.

(*f*) *Nitration of Pinoresinol Dimethyl Ether.*—Pinoresinol dimethyl ether (5 g) was refluxed with 50 ml conc. HNO_3 for 10 min (Robinson and Smith's conditions) and then poured into water. The crude product was filtered and shaken vigorously for several hours with separate batches of 5% aqueous KOH. Filtration and acidification of the filtrate precipitated the lactone (1·1 g) which crystallized from methanol as fine, pale yellow needles, m.p. 185–186 °C, $[\alpha]_D^{24·5}$ −93·7° (*c*, 2·2% in $CHCl_3$); light absorption in ethanol: $\lambda_{max.}$ 293, 323 mμ, ε 3900, 4150. Freudenberg and Dietrich (1953) record m.p. 180 °C (Found: C, 47·7; H, 4·1; N, 8·1; OMe, 17·5%. Calc. for $C_{14}H_{14}N_2O_9$: C, 47·5; H, 4·0; N, 7·9; OMe, 17·5%).

The alkali-insoluble material (3·9 g) was crystallized from ethanol to yield yellow crystals (3·8 g) of 4,5-dinitroveratrole, m.p. 129–130 °C (Found: C, 42·1; H, 3·4; N, 12·2; OMe, 27·8%. Calc. for $C_8H_8N_2O_6$: C, 42·1; H, 3·5; N, 12·3; OMe, 27·2%).

The mother liquors from this crystallization were chromatographed on alumina to yield more 4,5-dinitroveratrole and also 5,5′,6,6′-tetranitropinoresinol dimethyl ether in low yield (50 mg). This crystallized from ethyl acetate as pale yellow crystals, m.p. 221–221·5 °C (Found: C, 46·6;

H, 4·1; N, 9·6; OMe, 21·7%. Calc. for $C_{22}H_{22}N_4O_{14}$: C, 46·6; H, 4·0; N, 9·9; OMe, 21·9%); light absorption in ethanol: $\lambda_{max.}$ 322 mμ; ε 8300.

(*g*) *Nitration of Dinitropinoresinol Dimethyl Ether.*—6,6′-Dinitropinoresinol dimethyl ether (104 mg) on refluxing with conc. HNO_3 (1 ml) for 10 min, yielded crude 4,5-dinitroveratrole (67 mg, m.p. 125–130 °C) as the neutral product and the C_{14}-lactone from the alkali extracts as in (*f*).

(*h*) *Phenanthraphenazine Derivatives.*—5,5′,6,6′-Tetranitropinoresinol dimethyl ether (22 mg) was dissolved in 2 ml hot acetic acid and 1 ml conc. HCl. Zinc dust (1 *g*) was then added and the mixture filtered. A solution of phenanthraquinone (18 mg) in aqueous sodium bisulphite/sodium acetate was then added, the mixture boiled for 15 min, and then added to water. Crystallization of the precipitate from pyridine yielded the bisphenanthraphenazine as yellow crystals, which did not melt below 400 °C and at that temperature decomposed slowly in the melting point tube (Found: C, 75·4; H, 5·2%. Calc. for $C_{50}H_{38}N_4O_6$: C, 75·9; H, 4·8%).

The C_{14}-lactone (60 mg) was treated as above with the substitution of ethanol for acetic acid. The product was crystallized from glacial acetic acid to yield yellow crystals, m.p. 284 °C, with a yellow-green fluorescence in benzene (Found: C, 71·1; H, 4·9; N, 6·3%. Calc. for $C_{28}H_{22}N_2O_5$: C, 72·1; H, 4·7; N, 6·0%).

(*i*) *Action of Nitric Acid on the Lactone.*—The C_{14}-lactone (270 mg) was refluxed with 3 ml conc. HNO_3 for 4 hr. The diluted reaction mixture was extracted with ether after filtering off unchanged lactone, and from the ether was obtained 160 mg of bicarbonate-soluble material. Crystallization of this from methanol yielded a further small quantity of lactone. The mother liquors after evaporation were crystallized from water to yield pale yellow crystals (30 mg) of 5,6-dinitroveratric acid, m.p. 192–192·5 °C (Found: C, 39·8; H, 3·15; N, 9·5; OMe, 22·8%. Calc. for $C_9H_8N_2O_8$: C, 39·7; H, 2·9; N, 10·3; OMe, 22·8%). The literature (Klemenc 1912) gives m.p. 193 °C. The acid gives a white precipitate with ferric chloride solution and depresses the melting point of the lactone.

(*j*) *Ultraviolet Absorption Spectra of the Dinitroveratroles.*—These were synthesized by known procedures (Jones and Robinson 1917; Oxford 1926; Baker and Robinson 1929). The intermediate nitroguaiacols, being rather strongly acidic, were methylated satisfactorily by using diazomethane rather than the reagents previously employed. Chromatography on alumina in light petroleum, benzene, etc. was found satisfactory for separating the various products, the order of elution being 3,6-, 3,5-, and 3,4-dinitroveratroles.

The data obtained from the examination of the ultraviolet spectra in 95% ethanol is summarized in Table 1.

V. Acknowledgments

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THE CHEMISTRY OF ANTS

V. STRUCTURE AND REACTIONS OF DOLICHODIAL

By G. W. K. Cavill* and Hertha Hinterberger*

[*Manuscript received August 5, 1960*]

Summary

Dolichodial, isolated from *Dolichoderus acanthoclinea Clarki* (Wheeler), is shown to be α-(2-formyl-3-methylcyclopentyl)acraldehyde (I). The conversion of dolichodial into iridodial (II), and independently, into isoiridomyrmecin (X), establishes further relationships in the cyclopentanoid monoterpenes.

I. Introduction

The isolation of dolichodial and related compounds from various *Dolichoderus* and *Iridomyrmex* species of ants has been reported in Part IV of this series (Cavill and Hinterberger 1960). The determination of structure, and some reactions of dolichodial are now described.

Dolichodial, used in the present studies, has been extracted from *Dolichoderus acanthoclinea Clarki* (Wheeler). It is an almost colourless, strongly lachrymatory liquid, b.p. 96 °C/2 mm, $[\alpha]_D^{22\cdot5}$ −26°, and has been characterized by the formation of two derivatives, B and C, on treatment with 2,4-dinitrophenylhydrazine. Each of these products has been formulated as $C_{22}H_{22}N_8O_8$, whence the parent compound, $C_{10}H_{14}O_2$, has two carbonyl functions. Derivative B, m.p. 177 °C, has been converted into derivative C, m.p. 242 °C, on treatment with mineral acid (cf. Cavill and Hinterberger 1960).

II. Ultraviolet and Infrared Absorption Data

Dolichodial has an absorption in the ultraviolet region at 223 mμ (ε 6950 in water), indicating the presence of an α- or a β-monosubstituted, αβ-unsaturated, aldehyde group. Comparably, crotonaldehyde has a band at 223 mμ (water). The infrared spectrum of dolichodial shows a strong band at 1725 cm^{-1}, and a medium band at 2720 cm^{-1}, attributable to the carbonyl group of an aliphatic aldehyde (cf. Bellamy 1958). Further, a strong band at 1690–1700 cm^{-1} and a medium band at 1633 cm^{-1} are characteristic of the carbonyl group, and the carbon–carbon double bond, of an αβ-unsaturated aldehyde. Rasmussen (1948) reports absorptions at 1695 and 1639 cm^{-1} for α-methylacraldehyde. Thus dolichodial may contain an isolated aldehyde group, and an αβ-unsaturated aldehyde group.

Dolichodial is a highly reactive compound which darkens rapidly on standing, and this deterioration is accompanied by the appearance of a band at 1757 cm^{-1} in the infrared spectrum. This process is to be examined in more detail.

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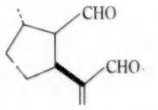
Typical carbonyl reagents do not appear to form simple derivatives with dolichodial. For example, the bis-derivatives with 2,4-dinitrophenylhydrazine, B and C, show an absorption ~360 mμ characteristic of a saturated 2,4-dinitrophenylhydrazone, but no absorption is shown ~380 mμ, which is characteristic of an αβ-unsaturated derivative. Attempts to prepare a disemicarbazone and a dithiosemicarbazone have been unsuccessful.

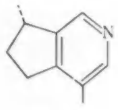
These difficulties emphasize a need for deactivation of the double bond, prior to the formation of carbonyl derivatives. Now, thiolacetic acid adds to the double bond of αβ-unsaturated aldehydes yielding the β-thiolacetate (Brown, Jones, and Pinder 1951). Dolichodial reacts with thiolacetic acid, giving a yellow oil with an absorption at 233 mμ (ε 5180), characteristic of the thiolacetate (cf. Cunneen 1947). Dolichodial thiolacetate is then characterized as a normal bis-2,4-dinitrophenylhydrazone, $C_{24}H_{26}N_8O_9S$.

III. Structure and Configuration of Dolichodial

Hydrogenation of dolichodial, in the presence of a palladium/barium sulphate catalyst, results in an uptake of one molar proportion of hydrogen, and the absorption at 223 mμ is reduced in intensity to 15% of that of the control. The reaction mixture gives an immediate precipitate with 2,4-dinitrophenylhydrazine, whence chromatography on alumina yields two products: a red derivative, m.p. 217 °C, and the major component, a yellow derivative, m.p. 228 °C. This compound does not depress the m.p. of an authentic specimen of iridodial bis-2,4-dinitrophenylhydrazone.



(I)



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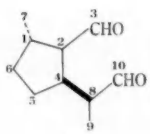
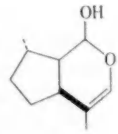


(II)



(III)

The conversion of dolichodial into iridodial (II) establishes the cyclopentanoid monoterpene skeleton, and as dolichodial has an absorption in the ultraviolet region at 223 mμ, it is assigned the monosubstituted acraldehyde structure (I). Dolichodial would have the same configuration as iridodial at the stable C_1 and C_4 centres.

On reaction with hydrochloric acid in acetic acid, each of the bis-2,4-dinitrophenylhydrazones, m.p. 217 and 228 °C, is converted into 1′,5-dimethyl-3,4-cyclopentenopyridine (IV), isolated as its picrate, m.p. and mixed m.p. 146 °C, with an authentic specimen (Cavill and Ford 1960). This transformation

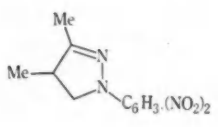
establishes that the newly isolated bis-derivative, m.p. 217 °C, is a stereoisomer of the known derivative, m.p. 228 °C, and differs in configuration at C_2 and/or C_8.

Ozonolysis of dolichodial, then decomposition of the ozonide with zinc dust in aqueous acetic acid, gives formaldehyde, isolated as the 2,4-dinitrophenylhydrazone. The remaining fragment/s have not been characterized satisfactorily, however, the isolation of formaldehyde, in 30% yield, confirms the α-acraldehyde structure (I), initially proposed on the basis of its light absorption data.

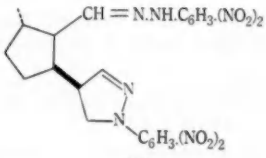
The infrared spectrum of dolichodial supports a 1,5-dialdehyde formulation (I), and in contrast to iridodial, there is little tendency for lactol tautomerism (cf. II⇌III). Indirectly, this observation would confirm the lactol structure (III) for iridodial, which requires enolization at C_8. This lactol (type III) was previously suggested as the tautomeric form of iridodial on conformational grounds (cf. Cavill and Ford 1960).

IV. Structure of Derivatives B and C

The bis-derivatives B and C, which have no absorption ~380 mμ for an αβ-unsaturated 2,4-dinitrophenylhydrazone, may be assigned pyrazoline structures on the basis of the acraldehyde structure (I) for dolichodial. The high intensity absorption ~360 mμ, shown by each of these derivatives, results from the 2,4-dinitrophenylhydrazone and the 2,4-dinitrophenylpyrazoline chromophores. Comparably, the pyrazoline (V) obtained from isopropenyl methyl ketone has an absorption at 360·5 mμ (Kawahara 1957). Thus derivative B is assigned structure (VI). It gives a green colour on treatment with bromine in chloroform, and this reaction is characteristic of pyrazolines (cf. Raiford and Peterson 1937). Derivative C, which is only slightly soluble in chloroform, gives a black crystalline solid. In contrast, no colour reaction has been observed for iridodial bis-2,4-dinitrophenylhydrazone.



(V)



(VI)

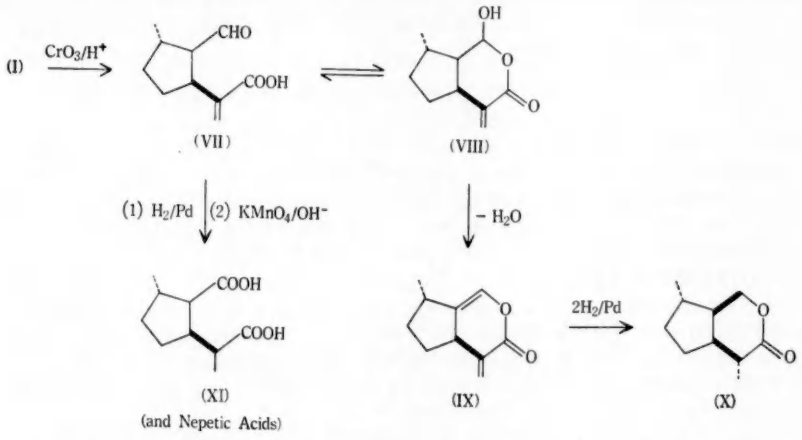
As derivative B is converted into derivative C, by the action of hydrochloric acid, and as each of these compounds has similar light absorption properties, it is possible that they are stereoisomers. Alternatively, the more complex infrared absorption spectrum of derivative C may indicate that it is a dimeric product, derived from B. Unfortunately, the highly insoluble nature of derivative C has prevented further investigation.

V. Oxidation, and Subsequent Transformations of Dolichodial

A mild oxidation of dolichodial with chromic acid/sulphuric acid in acetone (cf. Bowers *et al.* 1953) gives a yellow acidic oil, which has an absorption at 221 mμ (ε 20,400 in water), characteristic of an αβ-unsaturated acid. This product is

characterized as an orange 2,4-dinitrophenylhydrazone, $C_{16}H_{18}N_4O_6$, $\lambda_{max.}$ 370 mμ, which is soluble in sodium hydrogen carbonate solution. Thus the parent aldehydo-acid, $C_{10}H_{14}O_3$, is assigned structure (VII). A neutral fraction remained from the oxidation.

The aldehydo-acid (VII) readily absorbs 1·1M proportions of hydrogen, in the presence of a palladium catalyst, and the resultant aldehydo-dihydroacid/s then give a mixture of dicarboxylic acids, on oxidation with alkaline potassium permanganate solution. Paper chromatography indicates that this mixture contains the nepetalinic and nepetic acids. Esterification with diazomethane, followed by a microdistillation, and then saponification of a major fraction, yields a mixture of nepetalinic acids. These acids could not be resolved.



The neutral fraction, isolated during the original chromic acid oxidation of dolichodial, does not give a derivative with 2,4-dinitrophenylhydrazine. In the presence of a palladium catalyst, it absorbs 2·1M proportions of hydrogen, yielding isoiridomyrmecin, identical with an authentic specimen from *I. nitidus* (Cavill and Locksley 1957).

These oxidation and reduction sequences, which result in the conversion of dolichodial into isoiridomyrmecin (X), and separately, into the nepetalinic acids (XI), establish new relationships in the cyclopentanoid monoterpenes. Moreover, they support the assignment of structure (VII) to the aldehydo-acid, which is the primary oxidation product of dolichodial, whence the neutral product may be assigned structure (IX), resulting from a dehydration of the lactol form (VIII) of the aldehydo-acid.

VI. Experimental

Melting points are uncorrected. Light petroleum has b.p. 40–60 °C. Alumina refers to aluminium oxide, "Peter Spence, grade H." Solvent extracts were dried over anhydrous magnesium sulphate. Microanalyses are by Dr. E. Challen of this University and by Dr. K. W. Zimmerman and assistants, C.S.I.R.O. and University of Melbourne Microanalytical Laboratory. Infrared spectra are by Mr. I. Reece of this University.

(*a*) *Isolation of Dolichodial.*—Dolichodial has been obtained from the light petroleum extract of *Dolichoderus acanthoclinea clarki* as previously reported (Part IV loc. cit.). It is an almost colourless, lachrymatory liquid, b.p. 96 °C/2 mm, n_D^{23} 1·4792, $[\alpha]_D^{22\cdot5}$ —26° (*c*, 4·36 in benzene).

(*b*) *Spectroscopic Data.*—(i) The crude extract has an ultraviolet absorption at 223 mμ (in water). Freshly distilled dolichodial has an absorption at 223 mμ (ε 6950 in water), and comparably, acraldehyde has $\lambda_{max.}$ 212 mμ, and crotonaldehyde has $\lambda_{max.}$ 223 mμ.

(ii) Freshly distilled dolichodial has the following infrared absorptions (liquid capillary)* : 3400w, 3092w, 2930s, 2860s, 2720m, 1725s, 1690s, 1633m, 1460m, 1392sh, 1375m, 1332sh, 1305m, 1247m, 1228m, 1172m, 1140w, 1135w, 1113m, 1078m, 1042m, 1014w, 1008w, 952m cm^{-1}. In carbon tetrachloride : 3365w, 2930s, 2860m, 2820m, 2730m, 1757sh, 1725s, 1700s cm^{-1}.

A specimen of dolichodial, kept at 2 °C, for 4 weeks, has bands (liquid capillary) : 3470w, 2930s, 2860s, 2727m, 1745s, 1730sh, 1700s, 1640m, 1464m, 1380m, 1357w, 1330w, 1305m, 1285w, 1250m, 1173m, 1160m, 1132m, 1110m, 1054m, 1036m, 1016w, 988m, 950m, 812w, 723w cm^{-1}. In carbon tetrachloride : 2920s, 2860m, 2730m, 1757s, 1725m, 1700m cm^{-1}.

(*c*) *Biscarbonyl Derivatives.*—(i) The preparation of derivative B, m.p. 177 °C, $\lambda_{max.}$ 362 mμ (ε 38,000 in ethanol) and of derivative C, m.p. 242 °C, $\lambda_{max.}$ 358 mμ (ε 46,000 in chloroform), has been described previously (Part IV loc. cit.).

(ii) Attempts at the preparation of a disemicarbazone gave a colourless gum. The attempted preparation of the dithiosemicarbazone yielded a white amorphous solid, with absorptions at 275 mμ (ε 27,400), and at 300 mμ (ε 13,100), characteristic of an isolated, and an αβ-unsaturated, thiosemicarbazone (cf. Gillam and Stern 1957). The product could not be purified, and each attempt at recrystallization from ethanol resulted in a reduction in the intensity of the band at 300 mμ.

(*d*) *Reaction of Dolichodial with Thiolacetic Acid.*—Dolichodial (300 mg) was treated with thiolacetic acid (0·35 ml) at room temperature (18 hr), then the acid in excess was removed by distillation (at 40 °C/2 mm), and the residual oil extracted with ether. This extract was washed with saturated sodium hydrogen carbonate solution, water, and dried. Evaporation of the solvent gave the *thiolacetate*, as a viscous liquid (450 mg), $\lambda_{max.}$ 233 mμ (ε 5180 in ethanol).

The crude thiolester (300 mg) gave a liquid derivative, on treatment with 2,4-dinitrophenylhydrazine sulphate solution, which was extracted into chloroform, and purified by chromatography on alumina. *Dolichodial thiolacetate bis-2,4-dinitrophenylhydrazone* was finally isolated as yellow needles (30 mg), m.p. 93 °C, from benzene (Found : C, 48·1 ; H, 4·6 ; N, 17·9 ; S, 4·8%. Calc. for $C_{24}H_{26}N_8O_9S$: C, 47·7 ; H, 4·3 ; N, 18·6 ; S, 5·3%).

Treatment of crotonaldehyde, as described above, gave a yellow thiolacetate, $\lambda_{max.}$ 232 mμ (ε 6050), which was converted into the 2,4-dinitrophenylhydrazone, m.p. 93 °C. Brown, Jones, and Pinder (1951) report m.p. 95 °C.

(*e*) *Hydrogenation.*—Freshly distilled dolichodial (245 mg) in ethanol (7 ml) was hydrogenated in the presence of a palladium catalyst (100 mg, from 5% palladium oxide on barium sulphate) at r.t.p, and 38 ml of hydrogen (1·1M proportion) was consumed (30 min). Removal of the catalyst, and evaporation of the solvent, gave a pale yellow oil (250 mg), $\lambda_{max.}$ 223 mμ (ε 1080 ; that is, 15% of the intensity of the control).

An alcoholic solution of the reduction product (240 mg), on treatment with 2,4-dinitrophenylhydrazine reagent, gave an orange precipitate (562 mg), which was purified by chromatography on alumina. Derivative B (5 mg), m.p. and mixed m.p. 177 °C, and derivative C (15 mg), m.p. and mixed m.p. 242 °C, were eluted with benzene, then a red *compound* (39 mg), m.p. 217 °C, and a yellow compound (94 mg), m.p. 228 °C, were obtained. The latter product does not depress the m.p. of iridodial bis-2,4-dinitrophenylhydrazone (Found : C, 49·9 ; H, 4·5 ; N, 20·9%. Calc. for $C_{32}H_{34}N_8O_8$: C, 50·0 ; H, 4·6 ; N, 21·2%).

Each of the derivatives, m.p. 217 and 228 °C, was converted into 1′,5-dimethyl-3,4-cyclopentenopyridine, $\lambda_{max.}$ 262 and 269 mμ, and the base isolated as the picrate, m.p. and mixed m.p. 146 °C, with an authentic specimen (Found : C, 50·9 ; H, 4·0 ; N, 14·5%. Calc. for $C_{16}H_{16}N_4O_7$: C, 51·1 ; H, 4·3 ; N, 14·9%).

* Bands are characterized as s, strong ; m, medium ; w, weak ; sh, shoulder.

(*f*) *Ozonolysis.*—A mixture of ozone and oxygen was passed through a solution of dolichodial (430 mg) in dry ethyl acetate (30 ml), at —30 °C (15 min). The reaction mixture was slowly added (1 hr) to a cooled, well-stirred, suspension of zinc powder (2 g) in acetic acid (50 ml ; 50%). Evaporation of the solvent (at 40 °C/40 mm), then removal of the excess of zinc, gave a pale yellow solution, which was treated with an excess of 2,4-dinitrophenylhydrazine reagent. The gummy red product (775 mg) was purified by chromatography on alumina, the major constituent, formaldehyde 2,4-dinitrophenylhydrazone (151 mg), m.p. and mixed m.p. 165 °C, being eluted with light petroleum (Found : C, 40·3 ; H, 3·0 ; N, 26·4%. Calc. for $C_7H_6N_4O_4$: C, 40·0 ; H, 2·9 ; N, 26·7%).

Benzene eluted derivative B (10 mg), m.p. and mixed m.p. 177 °C, then benzene/chloroform (1/1) eluted derivative C (16 mg), m.p. and mixed m.p. 242 °C. The residual products, from the chloroform eluate, were rechromatographed giving a dark red compound (5 mg), m.p. 212 °C, as hexagonal plates from benzene. A second product (7 mg), m.p. 81 °C, was obtained as yellow needles from light petroleum/benzene. These products have not been identified.

(*g*) *Chromic Acid Oxidation, and Subsequent Reactions.*—(i) A solution of dolichodial (540 mg) in acetone (10 ml) was treated with chromic acid (2·5 ml of a standard solution, prepared by the method of Djerassi, Engle, and Bowers (1956)). The reaction mixture, after standing at room temperature (3 hr), was diluted with water, and then extracted with ether. An acidic product was extracted from the ethereal layer with sodium hydrogen carbonate solution (4 × 10 ml), and a neutral fraction (188 mg) remained, which had a positive reaction with 2,4-dinitrophenylhydrazine. This fraction was reoxidized with chromic acid (6 hr), and then worked up as described above. The neutral fraction, which now remained (100 mg, 18%), does not give a positive test for carbonyl compounds.

The combined sodium hydrogen carbonate extracts gave a yellow acidic oil (296 mg, 50%), which could not be crystallized. It has an absorption at 221 mμ (ε 20,400 in water). This acidic mixture, on treatment with 2,4-dinitrophenylhydrazine, gave an orange product, in 65% yield, which was purified via its sodium salt. This *2,4-dinitrophenylhydrazone* of the aldehydo-acid (VII) was finally isolated as orange needles, m.p. 196–197 °C, $\lambda_{max.}$ 370 mμ, from benzene (Found : C, 52·7 ; H, 5·0%. Calc. for $C_{16}H_{18}N_4O_6$: C, 53·0 ; H, 5·0%).

(ii) The aldehydo-acid mixture (210 mg) in ethanol (10 ml) was hydrogenated, as described above, 28 ml (1·1M proportion) of hydrogen being taken up (10 min). Removal of the catalyst, and evaporation of the solvent, gave a pale yellow acidic gum (210 mg), which showed no absorption between 215–260 mμ. Attempts at crystallization of these acid/s were unsuccessful, in addition, they did not give a solid derivative with 2,4-dinitrophenylhydrazine.

(iii) The aldehydo–dihydro–acid mixture (200 mg), from (ii), was oxidized with potassium permanganate (160 mg), in aqueous sodium hydroxide solution (5 ml ; 5%) at room temperature (18 hr). After working up the reaction mixture in the normal manner, an acidic oil (180 mg) was obtained, which on paper chromatography, employing an ethanol–ammonia–water (85 : 5 : 15) system, showed the presence of at least two nepetalinic acids, R_F values 0·63 and 0·62, and one nepetic acid, R_F 0·49. Reference compounds, run in the same chromatogram, were the *cis,trans*-nepetalinic acid, m.p. 82 °C, which has an R_F value 0·62, and the *cis,trans*-nepetic acid, which has R_F 0·49 (see Cavill and Ford 1960).

This acid mixture (100 mg) was esterified with diazomethane, and the major fraction, a colourless ester (30 mg), b.p. 90–100 °C/2 mm, separated by a microdistillation. Saponification of this ester fraction gave a colourless acidic oil (16 mg) (Found : equiv. wt, 100·0. Calc. for $C_{10}H_{16}O_4$: equiv. wt, 100·0).

(iv) The neutral product (60 mg), formed during the initial chromic acid oxidation of dolichodial, in (i), on hydrogenation, as above, consumed 20 ml (2·1M proportions) of hydrogen (15 min). A colourless oil (40 mg) was obtained, which on sublimation yielded isoiridomyrmecin, finally isolated as colourless needles, m.p. 56 °C, from light petroleum. The m.p. was undepressed on admixture with an authentic specimen from *I. nitidus* (Cavill and Locksley 1957).

VII. Acknowledgment

The authors thank the Commonwealth Bank of Australia (Rural Credits Development Fund) for a grant to aid this investigation.

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THE α-HYDROXYACIDS OF SHEEP BRAIN

By D. T. Downing*

[*Manuscript received October 5, 1960*]

Summary

The α-hydroxyacids of sheep brain are shown to consist of a series of straight-chain compounds having 14 to 26 carbon atoms. The acids were isolated from the total lipids of sheep brain and a gas chromatographic analysis was made of the isopropylidene derivatives of the diols obtained on reduction of the α-hydroxyacids. Analyses were also carried out on the mixture of hydrocarbons obtained on reduction of the diols.

I. Introduction

Klenk (1928*a*) and Chibnall, Piper, and Williams (1936, 1953) have shown that the phrenosin from brain contains long-chain α-hydroxyacids of 22, 24, and 26 carbon atoms. Klenk (1928*a*, 1928*b*, 1953) has also shown the presence of Δ-15 and Δ-17 unsaturated C_{24} α-hydroxyacids. Recently, the occurrence of α-hydroxystearic acid in phrenosin from beef spinal chord has been claimed (Skipski, Arfin, and Rapport 1959). The methods used by these workers for the isolation of the phrenosin fraction involved extensive recrystallization, and it is probable that minor and more soluble constituents were discarded. It was considered possible that the material thus overlooked might include branched-chain α-hydroxyacids similar to those which are so abundant in the wax from the wool of sheep (Horn *et al.* 1954 ; Downing, Kranz, and Murray 1960). This possibility has now been examined.

During the present work the publications of Kishimoto and Radin (1959*a*, 1959*b*) on the cerebrosides of rat brain appeared, and confirmed the view that knowledge of the fatty acids of cerebrosides was incomplete. They also suggested that branched-chain acids were present in these lipids. Kishimoto and Radin separated the cerebrosides from the remainder of the brain lipids by adsorption chromatography. The acids obtained on hydrolysis of the cerebrosides were esterified and separated by adsorption chromatography into hydroxylated and unhydroxylated methyl esters, which were each then resolved into saturated and unsaturated fractions. Gas chromatographic analysis showed that each of these four fractions contained normal compounds of 20 to 24 carbon atoms. There was evidence for the presence of acids of both longer and shorter chain-lengths, which were thought to be branched-chain compounds.

In the work reported here it has been shown that the α-hydroxyacids of sheep brain are normal compounds with chain-lengths ranging from 14 to 26 carbon atoms. The quantity of α-hydroxyacids found (0·25%) represents a concentration of 0·55% of phrenosin in the fresh brain, or nearly 6% in the brain lipids.

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II. Results and Discussion

In order that none of the α-hydroxyacids of brain might be overlooked it was decided to avoid the isolation of the cerebrosides, or the phrenosin fraction, and to work with the total fatty acids of brain. Accordingly, the whole brain lipids were extracted, hydrolysed, and the fatty acids separated. These were then hydrogenated, methylated, and reduced to the corresponding mixture of alcohols. From these the αβ-diols, arising from the α-hydroxyacids, were separated in the form of their isopropylidene derivatives. This mixture of ketals was then examined by gas chromatography on a non-polar stationary phase. The retention times observed were compared with those of a reference mixture consisting of the isopropylidene derivatives of αβ-diols derived from wool

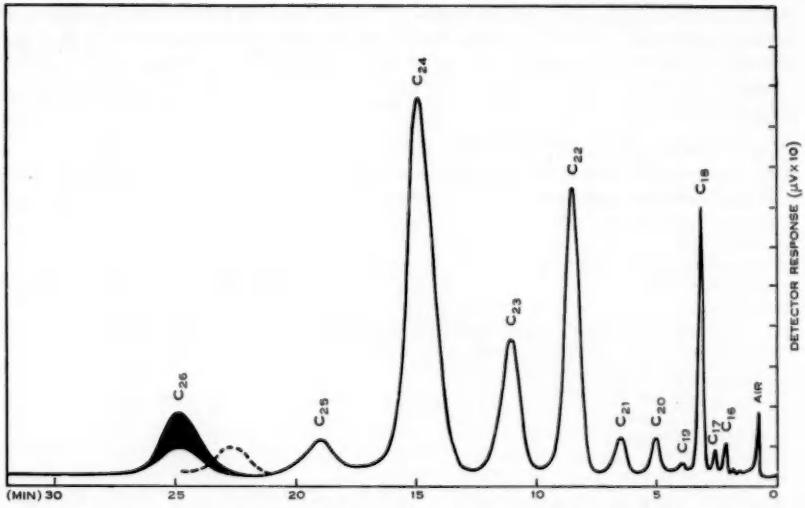

Fig. 1.—Chromatogram of the hydrocarbons derived from sheep brain α-hydroxyacids. Column temperature 240 °C, N_2 pressure 790 mm, charge 2·5 μl. The black area represents the peak increase obtained after addition of n-hexacosane to the mixture. The broken curve shows the position expected for a singly methyl-branched hydrocarbon of the iso series.

wax (Downing, Kranz, and Murray 1960). In this way it was possible to deduce the number of carbon atoms in each of the components of the mixture. In a plot of carbon number against the logarithm of retention time the points obtained for each of the ketals derived from brain, and those obtained for the straight-chain constituents of the wool wax ketals, fell on the same straight line. This suggested that the fraction from brain contained only straight-chain compounds. They ranged in chain-length from 14 to 26 carbon atoms, and some of the odd carbon-numbered constituents were present in quite large amounts. It is possible that some of the α-hydroxyacids do not arise from phrenosin, but there has been no report of such compounds being isolated from other fractions of nervous tissue.

The results are generally in agreement with those of Kishimoto and Radin who studied chromatographically isolated phrenosins.

Further evidence for the assignment of straight-chain structures has been provided by a study of the hydrocarbons derived from the α-hydroxyacids. The hydrocarbons, prepared by conversion of the αβ-diols to the di-iodides and reduction of these with lithium aluminium hydride, were examined by gas chromatography. A plot of the results showed a straight line in the carbon number-log retention time graph. When the hydrocarbon mixture was subjected to gas chromatography after admixture with a small proportion of authentic n-hexacosane there was an increase in the height of the peak assigned to the C_{26} hydrocarbon without any disproportionate broadening (Fig. 1). Finally, the hydrocarbons were chromatographed with a short column of Linde 5 Å Molecular Sieve in series with the main column. It had previously been shown that this system achieved quantitative absorption of normal hydrocarbons from the gas stream while hydrocarbons having a single methyl side chain were wholly unabsorbed (Downing, Kranz, and Murray 1960). In the present instance approximately 1% of the charge was unabsorbed in this system, the remaining 99% therefore being straight-chain material. A peak corresponding to a C_{24} hydrocarbon accounted for half of the unabsorbed material, and may represent either a branched-chain component or a small residue of unsaturated hydrocarbon.

TABLE 1

CHAIN-LENGTH ANALYSIS OF THE α-HYDROXYACIDS OF SHEEP BRAIN

Chain-length	Wt. % of Hydroxyacid Fraction	Chain-length	Wt. % of Hydroxyacid Fraction
n-C_{14}	<0·1	n-C_{21}	2·5
n-C_{15}	0·2	n-C_{22}	19
n-C_{16}	0·8	n-C_{23}	12·5
n-C_{17}	0·8	n-C_{24}	44
n-C_{18}	6·5	n-C_{25}	5·5
n-C_{19}	0·6	n-C_{26}	6
n-C_{20}	2		

The composition of the α-hydroxyacid mixture (Table 1) was calculated from the areas of the peaks in the chromatogram of the derived hydrocarbons. Analyses based on chromatograms of the ketal derivatives gave similar results, but were less accurate because of poorer resolution of the peaks. However, analysis of α-hydroxyacids by examination of the derived diol ketals in this way offers a rapid and convenient quantitative method for the study of these compounds, which evidently are widespread in biological systems (Deuel 1951).

No analysis has been made in the present work of the unsaturated α-hydroxyacids. Kishimoto and Radin (1959*a*, 1959*b*) showed that in the α-hydroxyacids of rat brain the unsaturated components comprise about 10% of the mixture, and have a chain-length distribution similar to that of the saturated members. The ketals of the αβ-diols derived from hydrogenated sheep brain acids melted

at 46 °C, while those from unhydrogenated acids melted at 43 °C, suggesting that the proportion of unsaturated compounds is small. In any one chain length the double bond apparently may occur in at least two different positions (Klenk 1953), so that examination of the unsaturated acids will require a clear-cut separation according to chain length prior to oxidation and analysis of the fission products.

III. Experimental

The magnesium trisilicate used was British Drug Houses Ltd. "Chromatographic Analysis Grade". It was washed with methanol, then water, dried for 4 hr at 250 °C, and shaken with 3% by weight of water. Light petroleum refers to that fraction boiling 60–80 °C.

(*a*) *Extraction of Sheep Brain.*—Brain (330 g) obtained from four freshly-slaughtered sheep was homogenized with four volumes of 96% ethanol. The mixture was refluxed for half an hour and filtered. The solids were refluxed with three successive portions of chloroform : methanol (1 : 1), the extracts washed with 30% aqueous ethanol and evaporated. The original ethanol extract of the homogenate was diluted with two volumes of water, extracted three times with hot chloroform, and the residue from evaporation of the extracts combined with the material from the chloroform : methanol extracts of the brain solids. The recovery of soft waxy solid was 32·95 g.

(*b*) *Hydrolysis of the Lipids.*—A portion of the lipids (18·84 g) was refluxed for 1 hr in an atmosphere of nitrogen with 100 ml benzene and 200 ml ethanol containing 2 g hydrogen chloride. A solution of 15 g KOH in 100 ml ethanol was then added and refluxing continued for a further hour. The mixture was diluted with 400 ml water, the unsaponifiable material extracted with three portions of hot benzene, and the alkaline layer treated with a solution of 20 g calcium chloride in 500 ml water. Most of the solvent was removed on a water-bath and the precipitated calcium salts collected, dried, powdered, and extracted three times with hot acetone to remove a small portion of remaining unsaponifiable material. The salts were refluxed for 3 hr in an atmosphere of nitrogen with 300 ml methanol containing 15 g HCl, the mixture diluted with 600 ml water and extracted with light petroleum, which was washed and evaporated to give 7·25 g methyl esters.

The combined unsaponifiable material weighed 5·90 g.

(*c*) *Preparation of the αβ-Diols.*—(i) A portion of the methyl esters (3·25 g) was hydrogenated by shaking with hydrogen and Adams's catalyst in ethyl acetate–ethanol solution, then recovered, taken up in ether, and reduced with lithium aluminium hydride to give 3·22 g alcohols showing no carbonyl absorption in the infrared spectrum. The product was taken up in acetone, treated with two drops of concentrated H_2SO_4, and after 2 hr made alkaline. The solvent was removed and the residue dissolved in light petroleum and chromatographed on magnesium trisilicate. Light petroleum eluted 0·17 g oil which solidified on standing at room temperature to a mixture of crystals and oil which on heating finally melted at 46 °C. In order to be sure that only ketals were present in this fraction the mixture was boiled down with ethanol containing one drop of conc. HCl. The liberated diols were chromatographed on magnesium trisilicate, from which no trace of material was eluted with light petroleum, benzene, or chloroform. Chloroform : ethanol (9 : 1) eluted 0·12 g diols. These were reconverted to the ketals with acetone containing a trace of H_2SO_4 and a solution in light petroleum percolated through magnesium trisilicate. The recovered material and the original ketal preparation were compared by gas chromatography and found to give identical elution patterns.

(ii) A further 52·6 g of methyl esters of brain acids were prepared as described above from 570 g sheep brain, omitting only the catalytic hydrogenation. The esters were chromatographed on magnesium trisilicate and the unhydroxylated esters eluted with light petroleum and benzene. The more polar material eluted with ether and ether/ethanol, was reduced with lithium aluminium hydride, the product treated with acetone/sulphuric acid as described above and chromatographed on magnesium trisilicate. Light petroleum eluted the ketals (1·55 g), m.p. (clearing) 43 °C. On gas chromatography these showed an elution pattern identical with that of the ketals prepared

as in (i). The non-polar stationary phase did not give sufficient resolution of saturated and unsaturated components to change the shape of the curves. Polar polyester stationary phases were not sufficiently stable at the high temperatures required to allow an examination of the unsaturated ketals.

(*d*) *Reduction of the αβ-Diols to Hydrocarbons.*—A sample of the αβ-diols derived from the hydrogenated α-hydroxyacids was reduced to the corresponding mixture of hydrocarbons by conversion to the di-iodides and reduction of these with lithium aluminium hydride as previously described (Downing, Kranz, and Murray 1960).

(*e*) *Gas Chromatography.*—The apparatus and methods used were as described previously (Downing, Kranz, and Murray 1960). The mixtures were chromatographed on Silicone Elastomer E301 (Griffin and George Ltd., London) supported on 10 times its weight of Celite 545 (60–85 mesh) using nitrogen as carrier gas. The most suitable conditions for separation of the ketals were a column temperature of 270 °C and a flow rate of 29·8 ml/min.

IV. Acknowledgments

The author is grateful to Mr. K. E. Murray for advice and for providing the facilities for gas chromatography and to Mr. Z. H. Kranz for carrying out the gas chromatographic analyses.

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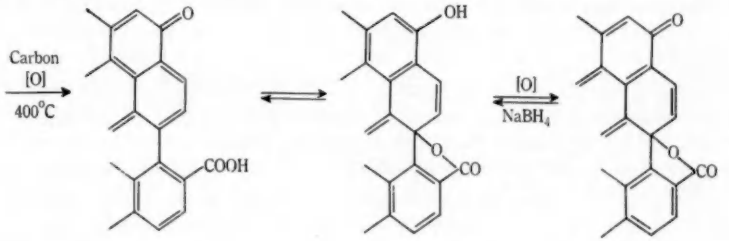
SHORT COMMUNICATIONS

THE REACTION OF CARBON BLACKS WITH SODIUM BOROHYDRIDE*

By V. A. Garten† and D. E. Weiss†

It has been shown (Garten, Weiss, and Willis 1957) that the acidity of carbon blacks is due to phenolic hydroxyls and two types of lactones. Those lactones which can be methylated by diazomethane were termed F lactones, whilst those that are not were termed N lactones.

It was also shown (Garten and Weiss 1955) that the acidity of a carbon that had been activated in air at 800 °C and cooled in nitrogen increased after reduction, and this was taken as evidence for quinones in these carbons. Studebaker *et al.* (1956) on the other hand suggested that since many carbon blacks are reduced by sodium borohydride they also contain quinones. However it was observed (Garten and Weiss 1957) that the acidity of these carbons did not change on reduction, from which it was concluded " that either quinone groups are reduced beyond the hydroquinone stage or that a group other than quinone must be responsible for the oxidation of the borohydride ". It has recently been realized, however, that this conclusion is not necessarily valid and that the following mechanism for the oxidation of a carbon by air at about 400 °C would reconcile Studebaker's work with our own and also with Hallum and Drushel's (1958) polarographic evidence for quinones.



(I) (II) (III)

It is now postulated that the primary step in the oxidation of the carbon black during its production by the channel or roller processes is the formation of carbonyl and carboxyl groups as in (I). These groups are unstable when interconnected by conjugated double bonds and, as in the phthalein dyestuffs, revert to the more stable F lactone tautomer (II). F lactones have been shown to be

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responsible for a major portion of the acidity of ink blacks. However, the hydroxyl of an F lactone may also be conjugated with respect to that of a second F lactone elsewhere on the layer plane and should therefore behave as a hydroquinone. Hydroquinone-like properties have been reported previously for low-temperature carbons (Garten and Weiss 1955). Hence some oxidation of these to (III) is likely and this would give rise to quinones and N lactones, which are also present in carbon blacks. Thus we find that in the highly oxidized L.F.C. and L.C.C. blacks the N lactone concentration is two to three times that of the F lactones. Mild reduction, e.g. with sodium borohydride, may produce (II) but the acidity would not alter as was observed. A comparison of the N lactone concentration of some carbon blacks (Garten, Weiss, and Willis 1957) with the consumption of hydrogen after treating the same type of black, but from different batches, with sodium borohydride (Studebaker *et al.* 1956) is given below and with the exception of the H.C.C. black shows fair agreement.

Type	N Lactones (m equiv/g)	% H Consumed from $NaBH_4$ (m-equiv/g)	Type	N Lactones (m-equiv/g)	% H Consumed from $NaBH_4$ (m-equiv/g)
H.C.C. ..	0·42	1·23	H.P.C. ..	0·25	0·29
L.F.C. ..	0·64	0·70	M.P.C. ..	0·23	0·23
L.C.C. ..	0·34	0·56	E.P.C. ..	0·36	0·29

We note that the determination of N lactones is only semiquantitative and tends to give too low values as indicated by the titration curves for carbon blacks which do not show a flat plateau for high pH values. The H.C.C. black is particularly bad in this respect and this may account for the big discrepancy between the two results.

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A CHEMICAL INTERPRETATION OF THE SEMICONDUCTIVITY OF AMORPHOUS CARBONS*

By D. E. Weiss†

It is well established by the detailed studies of Mrozowski and his school 1952, 1959; Kmetko 1951) and others (e.g. Hirabayashi and Toyoda 1952) that the electrical conductivity of different chars and carbons prepared by carbonization in the temperature range 600–800 °C is that of a *p*-type semiconductor which steadily reverts to a more metallic type conductivity at higher temperatures of carbonization. It is also established that the onset of electrical conductivity at carbonization temperatures of 600–700 °C is accompanied by a rapid loss in free electron spins as measured by electron spin resonance. This was accounted for by assuming " that above 700 °C the electrons which disappear from the π band are the same which become trapped by the spin centres and destroy their ability to show the paramagnetic absorption " (Akamatu, Mrozowski, and Wobschall 1959). However, no satisfactory reason could be advanced as to why the electrons should jump into the σ-traps only above a certain temperature.

It is possible to account for these facts in another way. X-Ray studies have shown that a considerable growth in the size of the layer planes occurs at carbonization temperatures exceeding 700 °C so the loss in free spins could be due to their combination during this growth. It is also well established that the chemical character of a carbon markedly changes at carbonization temperatures exceeding 700 °C. At lower temperatures the carbon is acidic due to chemisorbed acidic oxygen structures which decompose from 400 to 700 °C. Above 700 °C the carbon is predominantly basic. The basicity reaches a maximum at about 750 to 800 °C and becomes small at temperatures exceeding 1000 to 1200 °C. The oxygen content of a carbon falls from a value of about 2–3% when prepared at 800 °C to less than 1% at temperatures above 1000 °C. Recent studies have shown that at least some, if not all, of this oxygen is present as carbonyl and ether oxygen. Some of the ether oxygen is thought to be associated with the basicity of the carbon in chromene-like structures (for a recent review see Garten and Weiss 1957).

Because a carbonyl is an electron acceptor and an ether oxygen is an electron donor some electronic interaction between the two is to be expected within the

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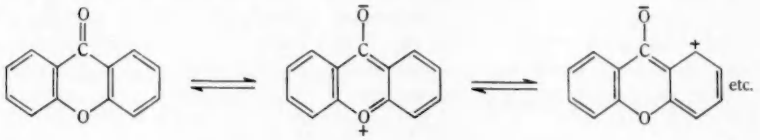
carbon. The simplest instance of this is xanthone which to some extent exists, even at room temperatures, as the following dipole:



This tendency to revert to a more aromatic structure is probably responsible for the extraordinary thermal stability of xanthone. At 850 °C this is reported to give a 10% yield of dibenzofuran (Orlow and Tistschenko 1930). An analogous interaction in carbons whose aromatization has proceeded far enough for through-conjugation to develop would give rise to the observed *p*-type semiconductivity since the proportion of the structure ionized, and hence the conductivity of the polymer, would be a function of temperature. As oxygen is lost during carbonization at temperatures exceeding 1000 °C these structures are destroyed and a less temperature-dependent conductivity arises. Thus the carbonyl group functions as an electron trap in a similar fashion to the carboxyl of a xanthene organic semiconducting polymer described recently (McNeill and Weiss 1959).

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A CAPACITANCE-OPERATED NULL DETECTOR FOR SURFACE BALANCES*

By F. H. C. Stewart†

The Wilhelmy plate technique has been used frequently in work on monomolecular films either in conjunction with an electromagnetic detecting system (cf. Padday 1957) or, more usually, with a torsion balance. In the latter case the movement of the plate is generally followed optically by means of a small mirror on the balance beam. Another possibility is to utilize the change in capacitance of a small variable condenser having its moving plate attached to the beam (Stewart 1960). The present work describes a simple detector unit based on this principle, which can be used conveniently with a normal laboratory surface trough for routine measurements on monolayers.

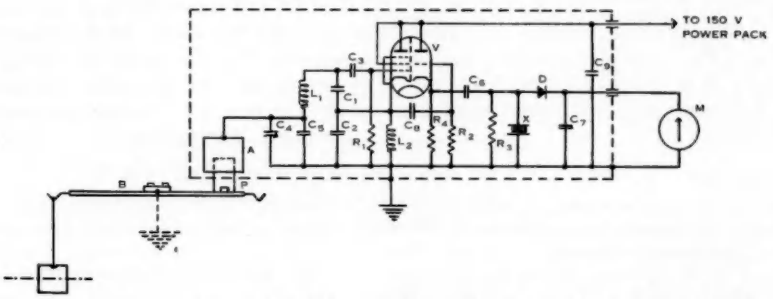

Fig. 1.—A diagrammatic sketch of the capacitance-operated null detector showing the approximate relative positions of the screened unit and the balance beam. Note that the aluminium plate P is earthed by way of the torsion wire and the frame of the balance. The various component values are : C_1, C_2, 0·001 μμF mica ; C_3, C_8, 100 μμF mica ; C_4, 3–30 μμF trimmer ; C_5, 47 μμF mica ; C_6, 22 μμF mica ; C_7, C_9, 0·1 μF paper ; R_1, 100 KΩ ; R_2, 1·5 MΩ ; R_3, 33 KΩ ; R_4, 10 KΩ ; L_1, 330 μH ; L_2, R.F. choke ; D, OA81 ; V, 6BL8 ; X, 1000 Kc/s crystal ; M, voltmeter.

There are numerous methods, of varying complexity and sensitivity, for the measurement and detection of small capacitance changes (Smith 1955). For the present purpose a quartz resonance method was selected, and a diagram of the final single-valve detector unit is shown in Figure 1, which also indicates the relationship of the detector to the torsion balance and Wilhelmy plate. The detector circuit is based on the four-valve dielectric constant apparatus of Buckingham, Harris, and Le Fèvre (1953).

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The transducer condenser consists of a piece of aluminium foil P (2 by 1·2 cm), attached to one end of the beam, and free to move vertically within a thin copper box A (2·5 by 3 by 0·3 cm). This system forms part of the tank capacitance of a Clapp oscillator built around the pentode section of the triode–pentode V. The Clapp circuit was chosen because of its good frequency stability with respect to variations in the internal valve impedances. The triode section of the valve is a cathode follower, which does not load the oscillator appreciably while providing a low impedance output to the germanium diode rectifier D and the D.C. voltmeter.

The operation and adjustment of the detector is straightforward. With the Wilhelmy plate in position in the surface the trimmer C_4 is adjusted until the oscillator frequency approaches the natural resonant frequency of the quartz crystal X. At resonance the latter acts as a very small shunt resistance so that the voltmeter registers a sudden dip in the rectified output voltage over a narrow range of capacitance. At all other frequencies the crystal constitutes a very high impedance. Some convenient reading on one side of this voltage crevice is selected as the zero reference point to which the meter is adjusted during a measurement by means of the torsion head. After the initial adjustment it is generally not necessary to alter the setting of the trimmer C_4 again.

The circuit is assembled in a small metal chassis (represented by the dashed line in Fig. 1), with the copper box A mounted on a Perspex block at one end. The unit is attached to the frame of the surface balance by brackets. It does not interfere with the normal manipulations of the balance, such as the hanging of weights on the beam for calibration, or the removal of equipment for cleaning.

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FIVE COORDINATION OF COPPER(II)*

By W. R. Walker†

This communication describes the isolation of a possible five-coordinated copper(II) complex of 4-methylpyridine with copper(II) acetylacetonate.

Graddon (1959) gave spectrophotometric evidence for the formation of five-coordinated complexes of substituted pyridines with copper(II) acetylacetonate. However, Tráill (1960) considers that Graddon's suggestion of the formation of a five-coordinated copper(II) complex is " not the only possible interpretation, nor the most probable one ". This criticism by Traill is not in accord with recent findings.

Such findings suggest that the five-coordination of copper(II) is unusual but not unknown. Llewellyn and Waters (1960) have shown that the structure of *NN'*-disalicylidenepropane-1,2-diamine copper(II) monohydrate contains five-coordinated copper with a pyramidal configuration. The structure of another five-coordinated copper(II) compound, terpyridyl copper(II) chloride dihydrate, was reported by Corbridge and Cox (1956). They contend that there is no doubt that its structure is essentially the same as that of the zinc compound—a distorted trigonal bipyramid. Further, Hall and Waters (1960) have shown that in *NN'*-disalicylidene-ethylenediamine copper, each copper atom is five-coordinated with a pyramidal arrangement of ligands. The same five-coordination of copper has been shown by Frasson, Bardi, and Bezzi (1959) in the dimer of copper(II)bis(dimethylglyoxime).

Because of Graddon's claim the above preparation was attempted. The ligand chosen as the most likely to form a stable compound with copper(II) acetylacetonate was 4-methylpyridine. According to Sacconi, Lombardo, and Paoletti (1958) this ligand has a high electron density on the nitrogen atom.

Experimental

Copper(II) acetylacetonate was prepared by the method of Jones (1959). The light blue crystalline solid was recrystallized from chloroform (Found : Cu, 24·4%. Calc. for $Cu(C_5H_7O_2)_2$: Cu, 24·3%).

Copper(II) acetylacetonate (1 g) was dissolved in B.D.H. 4-methylpyridine (about 15 ml) and refluxed for 30 min. On cooling the green solution, irregular hexagonal blue-green plates were obtained. These were dried between filter paper and transferred to a closed container. Washing even with cold dry benzene decomposed the complex. Analysis was carried out on single large crystals (Found : C, 53·9 ; H, 5·9 ; N, 3·9 ; Cu, 18·0%. Calc. for $CuC_{16}H_{21}O_4N$: C, 54·1 ; H, 6·0 ; N, 4·0 ; Cu, 17·9%). On standing in air it also slowly reverted to the pale blue colour of copper(II) acetylacetonate. Analysis was carried out on this residue, the carbon and hydrogen values being high due to the little 4-methylpyridine still present (Found : C, 47·3 ; H, 5·6 ; N, <0·5%. Calc. for $Cu(C_5H_7O_2)_2$: C, 45·9 ; H, 5·4%).

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The author is indebted to Dr. E. Challen (University of New South Wales), Dr. Zimmerman (C.S.I.R.O.) for microanalysis of C, H, and N, and to Dr. C. M. Harris (University of New South Wales) for his interest and assistance.

Note added in Proof.—Graddon and Watton (1960) discuss further evidence for the structure of 1 : 1 adducts of cupric β-diketone chelates with heterocyclic bases.

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THE EXTRACTION OF BERYLLIUM AND ALUMINIUM FROM AQUEOUS SULPHATE SOLUTIONS WITH DI-(2-ETHYLHEXYL) PHOSPHORIC ACID*

By R. W. Cattrall†

The experimental extraction equations are presented for the extraction of beryllium and aluminium from aqueous sulphate solutions with di-(2-ethylhexyl) phosphoric acid solution (EHPA) in kerosene. It is shown that under certain conditions beryllium can be separated from aluminium using this reagent. A structure is suggested for the beryllium complex.

A theory for the extraction of metal ions with weakly acidic chelating agents has been suggested (Morrison and Freiser 1957), and is expressed as the simplified equation (1).

$$D = K^* \left[\frac{[\mathrm{HR}]_o}{[\mathrm{H}^+]} \right]^n, \tag{1}$$

$$D = \frac{\text{concentration of metal in the organic phase}}{\text{concentration of metal in the aqueous phase}}$$

by taking logarithms

$$\log D = n[\mathrm{pH} + \log [\mathrm{HR}]_o] + \text{constant}. \tag{2}$$

A similar expression has been derived by Madigan (1959) using corrections for activity coefficients.

$$\log D = n[\mathrm{pH} + \log (c_{\mathrm{HA}})_o \cdot (y^{1/n}_{M^{n+}})_w] + \log K. \tag{3}$$

This equation has been experimentally verified for the extraction of copper, cobalt, and nickel with EHPA (Madigan 1959).

The experimental work described in the present paper was carried out in an attempt to verify equation (2) for the extraction of beryllium and aluminium from sulphate solutions with EHPA solution in kerosene.

Experimental

Solutions of beryllium sulphate and aluminium sulphate, 0·05M and 0·033M respectively, were prepared by dissolving the hydrated salts in water.

A 0·1M solution of acid form EHPA was prepared by dissolving the reagent in a kerosene fraction distilled at 180–210 °C. Nonanol (4% v/v) was added to the organic solution to prevent any third phase formation. The EHPA was found, by potentiometric titration with ethanolic KOH using an antimony electrode, to be 99·02% di-ester. Various pH values for the aqueous phase at equilibrium were achieved by adding calculated amounts of NaOH or H_2SO_4

* Manuscript received October 6, 1960.

† Australian Mineral Development Laboratories, Parkside, S.A.

before extraction. Equal volumes of aqueous and organic solutions were mixed thoroughly by rapid stirring at 30 °C for 2 hr to ensure that equilibrium was reached. After separation using ordinary laboratory separatory funnels, the organic phases were analysed for beryllium or aluminium and phosphorus, and the aqueous phases for beryllium or aluminium. The pH of each aqueous phase at equilibrium was measured using a precision pH-meter. It was considered that the amount of sodium extracted in the pH range studied would be negligible.

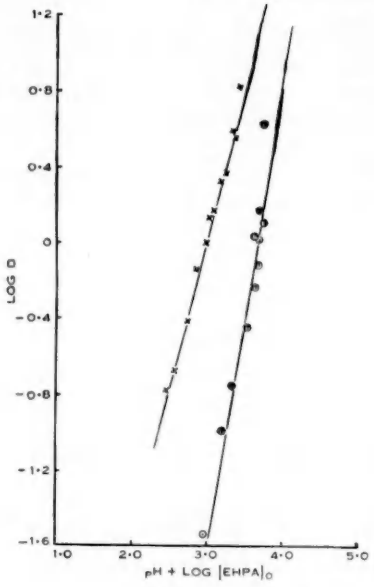


Fig. 1

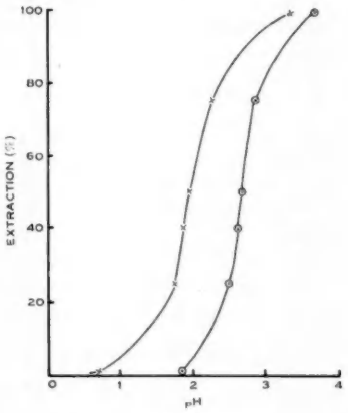


Fig. 2

Fig. 1.—Extraction of beryllium and aluminium by EHPA.
×—× Beryllium-slope 1·55; ⊙—⊙ aluminium-slope 2·38.

Fig. 2.—Extraction of beryllium and aluminium. Log $[EHPA]_o = 1$.
×—× Beryllium: ⊙—⊙ aluminium.

Discussion

(*a*) *The Extraction Equations.*—The results are graphically represented in Figure 1 by plotting log D against $[pH + \log[EHPA]_o]$. A straight-line relationship is obtained for both beryllium and aluminium. From Figure 1 the experimental extraction equations for beryllium and aluminium are obtained, and are shown below in equations (4) and (5).

Extraction equation for beryllium

$$\log D = 1 \cdot 55[pH + \log[EHPA]_o] - 4 \cdot 63. \quad (4)$$

Extraction equation for aluminium

$$\log D = 2 \cdot 38[pH + \log[EHPA]_o] - 8 \cdot 77. \quad (5)$$

The values for n obtained from Figure 1 are lower than the theoretical values of 2·0 and 3·0 for beryllium and aluminium respectively. This is to be expected because of the assumptions made in the derivation of the theoretical extraction equation (2).

The possibility of separating beryllium and aluminium by a solvent extraction process using EHPA may be evaluated as shown below. If the concentration of EHPA in the organic phase is kept constant at 10 mM/l, this value being chosen for convenience, then equations (4) and (5) may be written as

$$\log D = 1 \cdot 55[\mathrm{pH} + 1] - 4 \cdot 63, \tag{6}$$

$$\log D = 2 \cdot 38[\mathrm{pH} + 1] - 8 \cdot 77. \tag{7}$$

The extraction percentage E is related to D, the distribution ratio, as follows:

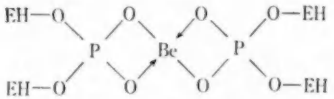
$$D = E/(100 - E). \tag{8}$$

Thus from equations (6), (7), and (8) the values of the pH for various values of extraction percentage can be calculated.

Figure 2 shows the extraction percentage of beryllium and aluminium for various calculated values of pH. It can be seen that by choosing the correct pH conditions it should be possible to separate beryllium and aluminium using a suitable number of extraction stages. At pH 2·2 the amounts of beryllium and aluminium extracted are 70 and 12% respectively, giving a separation factor which is defined as

$$D_{\mathrm{Be}}/D_{\mathrm{Al}} \quad \text{of } 17.$$

(*b*) *The Beryllium Complex.*—The beryllium complex was prepared by dissolving the stoichiometric weights of beryllium sulphate and EHPA in ethanol and boiling under reflux for several hours. The ethanol was then evaporated and the residue was boiled with water to remove any water-soluble impurities. The soap-like mass obtained was dissolved in benzene and poured into a water–acetone mixture. This procedure was repeated, yielding a soap-like compound which was shown by analysis to contain two EHPA molecules for each beryllium ion. The complex is probably a tetrahedral one formed by sp^3 hybridization of the beryllium ion.



EH = ethylhexyl

The compound as depicted above has two coordinate bonds from two oxygen atoms to the beryllium ion and two valence satisfying covalent bonds from the other two oxygen atoms. The suggested complex is neutral, with the EHPA groups placed tetrahedrally about the central beryllium ion.

This work is a part of a programme of investigation carried out on behalf of the South Australian Department of Mines, and the author wishes to thank the Honourable the Minister of Mines for permission to publish this paper.

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THE REMOVAL OF WATER FROM VOLATILE ORGANIC PRODUCTS OF OXYGEN-18 TRACER EXPERIMENTS*

By I. LAUDER† and I. R. WILSON†

The conventional methods of drying organic compounds (e.g. Morton 1938; Weissberger 1955) are not always immediately applicable to the drying of small quantities of alcohols etc. isolated from tracer experiments involving the use of ^{18}O-water. Several authors have reported work recently in which the application of a better drying technique would have been advantageous.

Bunton, de la Mare, and Tillett (1959) claim that diethyl sulphite hydrolyses entirely by sulphur-oxygen bond-fission although the ethanol isolated showed an apparent oxygen-18 at.% in excess of 0·034. The authors regard this as a slight enrichment and suggest that it is probably caused by incomplete drying of the ethanol. However, it could represent up to 15% carbon-oxygen fission.

Again Bunton *et al.* (1958) fractionated the methanol obtained from the hydrolysis of monomethyl phosphate in ^{18}O-water. Several samples were collected and two middle samples were used for isotopic analysis. The remaining samples were mixed with normal water and refractionated, two middle-samples again being analysed as a check on the efficiency of distillation. Rottenberg and Thürkauf (1959) investigated the oxidation of alcohols by oxygen gas in the presence of platinum using oxygen-18 as a tracer. In order to remove traces of oxygen-18 in the form of water from the products, the samples of alcohol (1 ml) were shaken in a 500 ml flask filled with "normal" carbon dioxide for 24 hr. This operation was repeated three to four times.

These reports have led us to give an account of an efficient, rapid method using anhydrous calcium sulphate for the drying of volatile substances which has been employed in this laboratory for the past 6 years. Hammond and Withrow (1933) reported the use of a similar principle in the second paper dealing with this drying agent, but for much larger quantities of material.

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Experimental

For the removal of 70–80 mg of water from a sample containing say 300 mg of methanol a vacuum system of the type illustrated by the line diagram in Figure 1 in used. Column A (0·8 cm i.d.) is packed with 7 g of calcium sulphate (40 mesh). Each of the columns I and II contains 0·6 g of calcium sulphate (40 mesh). The latter columns are constructed to the design shown in Figure 1, inset (*a*). All drying columns are wound with nichrome ribbon and are heated electrically to 180 °C and pumped for 15 min, or until no further water comes off, prior to use. All dryings are carried out with the columns at room temperature.

A vessel containing the alcohol to be dried is attached to the ground joint *B* and the alcohol is maintained at about 10 °C (so as to give a suitable vapour pressure) during the drying process. The vapour is pumped through tube *A* and is condensed in the U-bend *C* cooled in liquid air. The process is followed by means of the Pirani gauge at *D*.

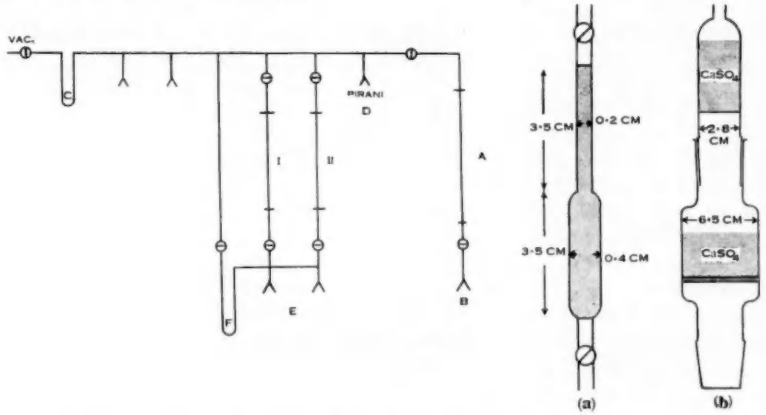

Fig. 1.—Diagram of vacuum-drying system using calcium sulphate.
Inset (*a*): Diagram showing design of column I.
Inset (*b*): Design of large column for absorbing 5 g of water.

During the drying a distinctly warm zone can be felt moving up column *A*. For a maximum recovery of alcohol, this warm zone should almost reach the top of the column, otherwise a small amount of alcohol remains adsorbed on the calcium sulphate. (A blank experiment is used to adjust the amount of calcium sulphate present if quantitative recovery is desired in a tracer experiment. Alternatively, more water may be pumped onto the column to make the warm zone move to the top.) If the absorptive capacity of the column is exceeded, a ring of ice forms 2–3 mm above the ring of alcohol in U-bend *C*. The alcohol in U-bend *C* is transferred to U-bend *F* and the process is repeated using column I and again using column II if desired. The alcohol in U-bend *F* is kept at about 10 °C during the drying stage.

If the water content of the alcohol is low the vessel containing the sample is attached at the ground joint *E* and columns I and II used directly.

Results

To test the efficiency of drying, two mixtures each containing 5·7 mg of water (at. % oxygen-18 in excess, 0·45) and 50 mg of methanol of normal isotopic composition were made up and then separated by passage over column I and then over column II. The pressure changes observed on the Pirani gauge are shown in Figure 2. In 3·5 min practically all the alcohol is recovered. The loss of alcohol amounted to 1·5% and experiment showed most of this occurred

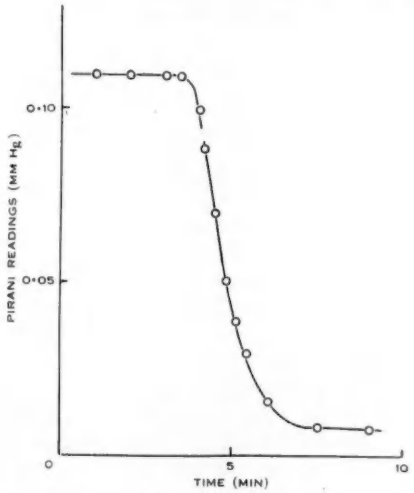

Fig. 2.—Pressure changes shown by the Pirani gauge at point *D* during the drying of methanol.

during passage through column II, when virtually no water is present to chase adsorbed alcohol off the calcium sulphate. The methanol showed a normal oxygen isotopic composition (cf. Lauder 1959 ; Lauder and Wilson 1959*a*, 1959*b* ; Lauder and Zerner 1959).

Larger columns of the type shown in Figure 1, inset (*b*), containing a total of 225 g of calcium sulphate (40 mesh) have been used to separate 5 g of ^{18}O-water from water–dioxan–methanol mixtures resulting from tracer experiments. The calcium sulphate in the larger columns takes up about 2·5%, while in the smaller columns it takes up about 1·5% by weight, before water can be detected escaping from the top of the columns. The water adsorbed can always be recovered and weighed and in this way the progress of drying may be followed.

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COUNTER-CURRENT DISTRIBUTION AND OTHER COMPARATIVE STUDIES ON THREE COMMERCIAL INSULINS*

By J. P. E. HUMAN† and S. J. LEACH†

The conditions under which insulin is extracted from pancreas are known to vary and this may be a reason for variations in the chemical and physical properties of the crystalline hormone. Harfenist and Craig (1952) have already shown that certain insulins contained one major and at least two minor components and that their heterogeneity varied with their source. The present authors have observed significant differences in solubilities of zinc insulins in the pH range 7 to 9. It is possible that preparations might differ also in other respects such as enzyme digestibility and this could account for conflicting reports on the susceptibility of zinc insulins to digestion by trypsin (Laskowski, Leach, and Scheraga 1960). Beef zinc insulins from three sources have therefore been compared with respect to homogeneity and trypsin digestibility. In addition, the u.v. absorption spectra of the insulins and their purified components have been characterized.

Counter-current distribution was carried out at 23 ± 2 °C with a Quickfit and Quartz 100 tube (25 ml per phase) automatic machine using the solvent system described by Harfenist and Craig (1952), namely, 2-butanol/1% dichloroacetic acid. Both were A.R. substances and were fractionally redistilled. For each distribution 1 g insulin was scattered in the first four tubes and extractions continued beyond 100 transfers by recycling the top phases. After 250, 500, 750, and 1000 transfers, the lower phases were analysed by measuring their absorption at 276 mμ. Figure 1 shows the distribution patterns for the three insulins after 750 transfers and the dotted lines indicate the theoretical curves for ideal behaviour.

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In addition to the main component A, each insulin contained the faster moving minor component B and evidence of a slower component C, suggested by the divergence of the left side of the curves from the theoretical shape. However, based upon the patterns after 1000 transfers, the amount of component B in the sample of Lilly insulin was only 18% as compared with 30% reported by Harfenist and Craig (1952) for an earlier batch. In addition, the mean partition ratios for components A and B were substantially lower in the present work, namely, 0·37 and 0·46 instead of 0·49 and 0·59. The insulin from the Commonwealth Serum Laboratories was the most homogeneous preparation, containing 5% or less of component B and it is therefore to be preferred for chemical work on protein structure.

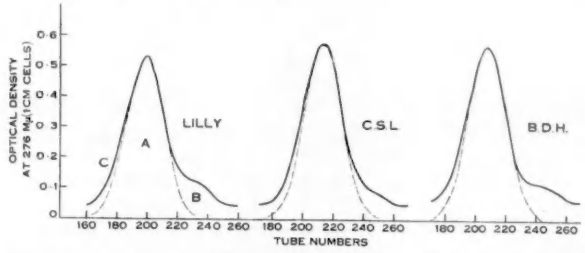

Fig. 1.—Counter-current distribution patterns of three commercial insulins after 750 transfers in the system 2-butanol/1% aqueous dichloroacetic acid.

The material from peak B of the Lilly sample contained 4·5 amide groups per mole whilst peak A and the original insulin contained 5·7 groups per mole.* This supports earlier suggestions that component B may have suffered deamidation during the commercial extraction process. In spite of this, the biological activity of component B was not significantly different (at the 0·01 probability level) from either A or the original insulin. The activities of the three commercial insulins before extraction are shown in Table 1 and are closely similar.

The u.v. absorption spectra of the three insulin samples were determined in 0·05% solution at pH 1·7 in 0·025N HCl, before and after counter-current distribution. The positions of the tyrosine peak at 275 mμ and shoulder at *c.* 281 mμ, and the phenylalanine shoulders at *c.* 252, 258, 264, and 268 mμ were the same in each case. Extinction coefficients ($E_{1\,cm}^{1\%}$) were measured at the 275 mμ maximum. To minimize the light-scattering contribution due to protein aggregates and suspended particles of $>10\mu$ diameter, the protein solutions and diluents were filtered through G5M Jena sintered filters. In spite of this, values of $E_{1\,cm}^{1\%}$ had to be corrected for variable amounts of light-scattering by a linear extrapolation from the 360–310 mμ region down to the peak at 275 mμ. The correction varied from 2 to 4%. This procedure slightly underestimates the scattering contribution since the latter follows a λ^4 law. However,

* Kindly determined by Dr. J. H. Bradbury. Standard deviations were ±0·2 amide groups per mole.

a more refined correction is not warranted since optical density measurements are accurate to no more than $\pm 2\%$. Values of $E^{1\%}_{1\,cm}$ are shown in Table 1 and it appears that the three insulins do not differ significantly from each other or from the samples after countercurrent separation. The extinction coefficient for component B also falls within the same range. The mean value for all determinations was $11{\cdot}0 \pm 0{\cdot}2$. Literature values are scattered around this figure; however, conditions of measurements and methods of calculations are not always given.

TABLE 1

PROPERTIES OF THREE COMMERCIAL INSULINS

Insulin	Activity* (units/mg)		Zinc		Extinction Coefficient ($E^{1\%}_{1\,cm}$)
	Mean	Limits ($P=0{\cdot}95$)	Content† (%)	Purity‡ (%)	
B.D.H. 2189 (international sample)	22·5	20·4–24·8	0·39	94·4	11·0
B.D.H. (peak A) after c.c.d.‖ ..	19·9	17·8–22·3	—	93·6	11·3
Lilly 535, 664	19·8	18·2–21·6	0·49	95·9	10·9
Lilly (peak A) after c.c.d. ..	22·3	21·2–23·4	—	93·8	11·0
Lilly (peak B) after c.c.d. ..	20·5	19·5–21·6	—	—	—
C.S.L., A1	25·1	22·6–27·9	0·55	92·9	11·0
C.S.L. (peak A) after c.c.d. ..	—	—	—	94·8	10·9

* Mouse-convulsion method, using 200 mice per assay. Between 1 and 9 assays were required to provide the limits shown.

† Theoretical zinc content for 1 atom per 11,500 g is 0·56%.

‡ Purities of dry samples, based on Kjeldahl-nitrogen, assuming 15·88% nitrogen for pure insulin. Glucagon contributes to the nitrogen content but is present only to the extent of 0·5% by weight in the Lilly sample.

‖ Counter-current distribution.

Trypsin digestions were carried out using 0·5% solutions of each of the three insulins dissolved in ammonium acetate buffer at pH 8 and ionic strength 0·10. To these were added sufficient trypsin (0·2% from Worthington Biochemicals Corporation, New Jersey, dissolved in 0·025N HCl and containing 0·01M calcium chloride as stabilizer) to make the final solution 0·01% with respect to enzyme. Digestions were allowed to proceed for 48 hr at 23 °C. Controls containing all components except trypsin were also set aside. The six solutions were then adjusted to pH 5·4 and the unchanged insulin and "core" material removed by centrifugation. The u.v. absorption at 275 mμ of the supernatant liquor of the three controls showed that 99% of the original insulin had been precipitated. On the other hand, the isoelectric precipitation of the digested insulins left supernatant solutions with appreciable u.v. absorption. All three spectra were characteristic of tyrosine-containing solutes and were indistinguishable from one another in form. The optical densities at 275 mμ were compatible with splitting of about 60% of the B22–B23 arg–gly bonds of

the insulin B chain, resulting in the liberation of a soluble fragment containing one of the four tyrosyl residues in the protein.

The C-terminal octapeptide sequence of the B-chain of insulin contains two phenylalanyl residues as well as the tyrosyl group already referred to. The identity of the solublized insulin fragment is demonstrated by the fact that there are four shoulders on the absorption curve which are characteristic for phenylalanine and which are more pronounced that in the insulin spectrum.

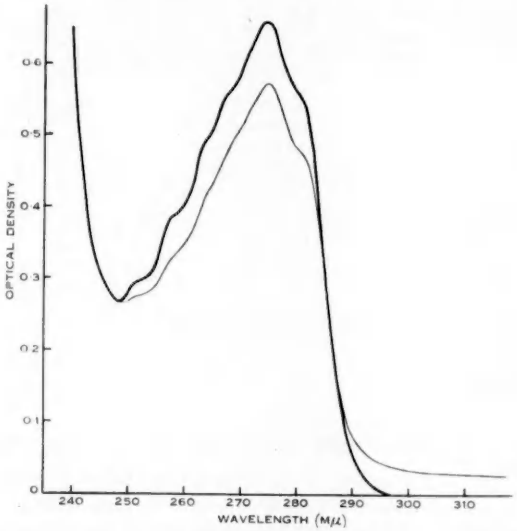

Fig. 2.—*Thick* (*upper*) ***curve:*** Absorption spectrum of the peptide gly.phe.phe.tyr.thr.pro.lys split out from insulin by tryptic digestion. *Thin* (*lower*) ***curve:*** Absorption spectrum of the original insulin. The concentrations were adjusted to give comparable optical densities.

Figure 2 shows the absorption spectrum of the peptide and also that of the original insulin for comparison. The identity of the peptide and the presence in solution of alanine from the terminal B30 position in all three cases was confirmed by paper chromatography as described by Laskowski, Leach, and Scheraga (1960).

It therefore appears that all three commercial insulins are susceptible to hydrolysis by trypsin at the B22–B23 arg–gly bond as well as the B29–B30 lys–ala bond. Nor do they appear to differ significantly with respect to their biological activity or u.v. absorption characteristics in the 240–300 mμ region. There are however significant differences in their counter-current behaviour which show that the sample from the Commonwealth Serum Laboratories (C.S.L.) is the most homogeneous.

The authors are indebted to Dr. O. K. Behrens of the Eli Lilly Co., Indianapolis, for gifts of crystalline insulin and to Mr. M. R. Hinton and Mr. A. H. Mengoni of the Commonwealth Serum Laboratories, Melbourne, for gifts of insulin and for their kind co-operation in carrying out many biological activity tests.

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HODGKINSINE, THE ALKALOID OF *HODGKINSONIA FRUTESCENS* F. MUELL.*

By E. F. L. J. ANET,† THE LATE G. K. HUGHES,‡ and E. RITCHIE‡

Following the observation by Webb (1949) that positive results were obtained in spot tests for alkaloids from the leaves of *Hodgkinsonia frutescens* F. Muell. (Order Rubiaceae), a shrub growing in the coastal and tableland regions of tropical Queensland, an extraction of the leaves has been undertaken. A crude amorphous alkaloid fraction was readily obtained but initially all attempts to isolate pure crystalline constituents were fruitless. However, subsequently, during one attempted purification by chromatography on alumina with benzene, an operation which had been performed many times previously, a few crystals were observed to form in the eluate. By using these as seeds, no difficulty was then experienced in crystallizing the major portion of the alkaloid fraction from benzene. The substance so obtained, after drying at room temperature and pressure, analysed for $C_{28}H_{32}N_4$. On drying at 80 °C/1 mm, it slowly lost benzene of crystallization, but the parent amorphous substance, hodgkinsine, $C_{22}H_{26}N_4$ (analyses and molecular weight), was more readily obtained by dissolving the crystals in dilute acid, shaking with ether, to remove the liberated benzene, and regenerating the base with alkali. All attempts to crystallize the alkaloid from any solvent other than benzene failed, but the crystalline benzene-solvated form was readily obtained.

Hodgkinsine, which was optically active and had pK_a values of 8·45 and 6·45 (in 50% ethanol), contained two *N*-methyl groups. At least one of the other two nitrogen atoms was present as an imino group since the i.r. spectrum had a band at 3320 cm^{-1} (Nujol). The spectrum also revealed the presence of an *o*-disubstituted benzene nucleus (bands at 740, 750 cm^{-1}).

* Manuscript received August 10, 1960.

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‡ Department of Organic Chemistry, University of Sydney.

Experimentally, the alkaloid was a peculiarly unsatisfactory substance. Numerous attempts to prepare crystalline salts, or derivatives, or degradation products by oxidation, reduction, or Hofman degradation were unsuccessful. By heating the alkaloid with zinc dust, there was obtained a small amount of oil which was presumably a mixture of indole derivatives, since a positive test was obtained with Erlich's reagent in the cold.

Hodgkinsine is isomeric with calycanthine, the structure of which has recently been elucidated (Hamor *et al.* 1960; Woodward *et al.* 1960) and is probably closely related to it, at least biogenetically. They have similar u.v. spectra (hodgkinsine: $\lambda_{max.}$ 232, 252, 310, 326 mμ, log ε 4·59, 4·48, 4·02, 3·93, respectively, in ethanol, scarcely different in 4% ethanolic hydrochloric acid; calycanthine: $\lambda_{max.}$ 252, 310 mμ, log ε 4·26, 3·80, respectively, in acetonitrile, $\lambda_{max.}$ 252, 311 mμ, log ε 4·5, 3·8, respectively, in dioxan), but calycanine, the very characteristic degradation product of calycanthine, could not be obtained from hodgkinsine. However, it is suggested that the formation of hodgkinsine, like that of calycanthine, involves the ββ′-oxidative coupling of *N*-methyltryptamine.

Experimental

Melting points are uncorrected. Analyses were performed by Dr. J. E. Fildes of this Department.

(*a*) *Extraction of Hodgkinsine.*—A dried mixture of the milled leaves (10 kg) and lime was exhausted with ether at room temperature and the combined extracts evaporated to about 3000 ml. The concentrate was shaken with 2N HCl (200 ml portions) until alkaloid was no longer extracted. The acid-extract was filtered and basified with ammonia. The precipitated alkaloid was collected, washed thoroughly with water, and dried. The crude alkaloid (60 g) was finely powdered and shaken with ether. After filtering from a small amount of insoluble material, the ether was evaporated and the residue crystallized from benzene with the aid of seeds obtained as described above. After three recrystallizations, colourless needles, m.p. 128 °C, were obtained (Found: C, 79·0; H, 7·5; N, 13·1%. Calc. for $C_{28}H_{32}N_4$: C, 79·2; H, 7·6; N, 13·2%). Amorphous solvent-free hodgkinsine was obtained by drying at 80 °C/1 mm or by dissolving the crystalline material in dil. HCl, removing the liberated benzene with ether, basifying the aqueous solution, extracting the base with ether, and evaporating the ether, finally, at 1 mm. The substance had $[\alpha]_D^{23}$ +60° (~, 1·0 in 0·3N HCl) (Found: C, 75·9; H, 7·5; N, 16·0; (*N*)CH_3, 13·7%; mol. wt., 360 (ebullioscopic in ethanol). Calc. for $C_{22}H_{26}N_4$: C, 76·2; H, 7·6; N, 16·2; 2×(*N*)CH_3, 16·7%; mol. wt., 346).

The authors are grateful to Dr. L. J. Webb, C.S.I.R.O., Brisbane, for the plant material, to Dr. J. R. Price for the spectroscopic data, and to the Commonwealth Research Grant Committee for the award of a studentship to one of them (E.F.L.J.A.).

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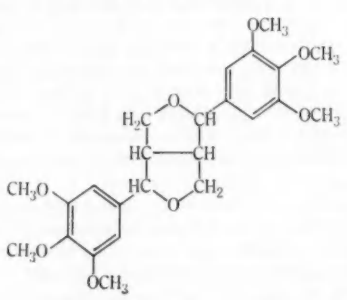
THE OCCURRENCE OF LIRIORESINOL-B DIMETHYL ETHER IN *EREMOPHILA GLABRA* R.Br.*

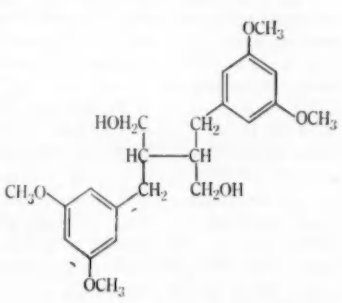
By P. R. Jefferies,† J. R. Knox,† and D. E. White†

Extraction of the leaves of *Eremophila glabra* R.Br. collected from Rottnest I. consistently afforded a colourless crystalline compound, $C_{24}H_{30}O_8$, which formed a dibromide, $C_{24}H_{28}O_8Br_2$. Only a trace of the same substance was obtained from the plant collected on the sand dunes of Rockingham, south of Perth. Six of the oxygen atoms were identified as methoxyl groups and the compound showed no infrared absorption corresponding to the presence of hydroxyl or carbonyl groups. Hence, it was considered likely that the remaining two oxygen atoms were attached by cyclic ether linkages, probably in a lignan of the 3,7-dioxabicyclo[3.3.0]octane type.

This has been confirmed by the well-established degradation technique used on this group of compounds, namely, the action of nitric acid on the dibromides (Erdtman and Gripenberg 1947 ; Hearon and McGregor 1955). The product in this case, 4-bromo-5,6-dinitropyrogallol trimethyl ether, indicated the structure (I) for the compound $C_{24}H_{30}O_8$.



(I)



(II)

The presence of benzyl ether linkages was demonstrated by hydrogenolysis, with sodium in ethanol, which afforded the diol (II). The loss of the central methoxyl groups would be expected for a pyrogallol trimethyl ether under these conditions as illustrated by the behaviour of elemicin (Semmler 1908), pyrogallol trimethyl ether (Thoms and Siebeling 1911) and 5-propylpyrogallol trimethyl ether (Sonn and Scheffler 1924). Permanganate oxidation of (II) yielded the expected 3,5-dimethoxybenzoic acid.

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At the time the structure of the lignan became clear, Dickey (1958) described the isolation of a diglucoside, liriodendrin, from *Liriodendrin tulipfera* L. Dependent on the methods used, three isomeric aglycones lirioresinol A, B, and C were obtained by hydrolysis of the glucoside. Dickey concluded on the basis of infrared comparison with synthetic (±)-syringaresinol (Freudenberg and Dietrich 1953) that the aglycones were stereoisomeric with the synthetic compound. Further degradation was shown to be analogous to that of syringaresinol although comparison with authentic samples was not made. Since then Pearl, Beyer, and Dickey (1958) have isolated from the spent sulphite liquor of aspen, three compounds of which one appears to be a further isomer of lirioresinol.

The identity of our material with lirioresinol-B dimethyl ether has been established by comparison with an authentic sample provided by Dickey.

The *cis*-fusion of the tetrahydrofuran rings is established by the optical activity of the diol (II). The alternative *trans*-fusion would be expected to lead to a *meso*-diol. Equilibration under the hydrogenolysis conditions would lead to racemization.

Experimental

Microanalyses were carried out by the C.S.I.R.O. Microanalytical Laboratory at the University of Melbourne.

Melting points were determined in open Pyrex capillaries and are uncorrected.

Optical rotations were measured in chloroform in 1 dm tubes at room temperature unless otherwise stated.

(*a*) *Extraction.*—The leaves and terminal branches (4·4 kg) collected at Rottnest I. were extracted with methanol (4 × 8 l.) and the methanol evaporated. The oily residue was extracted with ether and the solution washed successively with dil. HCl, 2·5% NaOH, and water. Evaporation of the solvent gave a green tar which was washed with light petroleum and crystallized from methanol yielding prisms, m.p. 122–123 °C (18·8 g), $[\alpha]_D$ +45·8° (*c*, 2·00 in 2 dm tube) (Found: C, 64·6; H, 6·8; O, 28·4; mol. wt., 423 (Rast); OCH_3, 40·8%. Calc. for $C_{24}H_{30}O_6$: C, 64·6; H, 6·8; O, 28·7; mol. wt., 446·5; 6 × OCH_3, 41·7%). There was no depression of melting point on admixture with a sample of lirioresinol-B dimethyl ether and the samples showed identical infrared spectra.

(*b*) *Bromination.*—The neutral material (I) (516 mg) was dissolved in carbon tetrachloride (22 g) and cooled to —4 °C. Bromine (330 mg) was added portionwise and the solution stood at 0 °C for 24 hr. After washing with saturated bicarbonate solution and evaporating to dryness a yellow solid was obtained which crystallized from methanol as needles, m.p. 157–157·5 °C, $[\alpha]_D$ —52·9° (*c*, 1·93) (Found: C, 47·6; H, 4·6; Br, 26·6%. Calc. for $C_{24}H_{28}O_6Br_2$: C, 47·7; H, 4·7; Br, 26·5%).

(*c*) *Nitration.*—The dibromide (5·0 g) was added in small portions to nitric acid (50 ml) in a flask which was vigorously shaken. The solution became a deep violet colour which after shaking for 24 hr had turned green and precipitated a solid. The mixture was heated on a steam bath for 1½ hr and cooled before water (200 ml) was added. Recrystallization of the precipitate from methanol gave 4-bromo-5,6-dinitropyrogallol trimethyl ether, m.p. 134–135 °C, alone or mixed with an authentic sample prepared by the method of Kohn and Grün (1925).

The filtrate was carefully neutralized with sodium bicarbonate to pH 6, and then evaporated to dryness under reduced pressure. A very small amount of oil was obtained from the residue by continuous ether extraction but failed to crystallize.

(*d*) *Hydrogenolysis of Lirioresinol-B Dimethyl Ether.*—The dimethyl ether (1·58 g) was dissolved in ethanol (25 ml) and sodium (7 g) added in small portions to the solution on a steam-bath over a period of 7 hr. The mixture was cooled, excess ethanol added, and the solution steam distilled before extracting with ether (3 × 30 ml). Evaporation of the extract gave a yellow oil (1·28 g)

which partially crystallized. The oil was washed with a little ether and the insoluble residue recrystallized from light petroleum/chloroform giving needles, m.p. 97·5–98·5 °C, $[\alpha]_D$ −20·4° (*c*, 0·85) (Found: C, 67·7; H, 7·7; O, 24·6; OCH_3, 31·5%. Calc. for $C_{22}H_{30}O_6$: C, 67·7; H, 7·7; O, 24·6; $4\times OCH_3$, 32·0%).

The infrared spectrum measured in carbon tetrachloride using a calcium fluoride prism showed absorption at 3638 cm^{-1} ($\varepsilon_{max.}$ 110) due to two primary hydroxyls.

(*e*) *Oxidation of the Diol* (*II*).—The diol (870 mg) was dissolved in acetone (40 ml) and treated with finely powdered potassium permanganate (4·0 g) with stirring. The mixture was allowed to stand for 16 hr and refluxed for a further 3·5 hr. The acetone was evaporated and the residue treated with excess sodium bisulphite solution. The solution was extracted with ether and the ether washed with 2·5% NaOH. The alkaline extract after acidification, extraction with ether, and evaporation of the solvent gave a red solid (300 mg) which recrystallized from aqueous methanol as prisms, m.p. 181–182 °C. There was no depression of melting point on admixture with an authentic sample of 3,5-dimethoxybenzoic acid and the infrared spectra were identical.

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THE ACTION OF METHYLMAGNESIUM IODIDE ON SOME CHLOROMETHYL SUBSTITUTED ANTHRACENES*

By F. H. C. Stewart†

Badger and Cook (1939) have drawn attention to the high reactivity of halomethyl groups in the *meso*-positions of anthracene and related polynuclear aromatic hydrocarbons. Thus, 9-chloromethylanthracene dissolves in warm methanol to form 9-methoxymethylanthracene with elimination of hydrogen chloride (Stewart 1960). A further example of this enhanced reactivity has now been observed during work on the synthesis of surface active anthracene derivatives (Stewart loc. cit.).

Addition of a benzene solution of 9-chloromethylanthracene (I; R=H) to methylmagnesium iodide at room temperature gave 1,2-di-9-anthrylethane (II; R=H) in good yield. This compound was accompanied by a small amount of its "photodimer" (IV; R=H), which has been described by Roitt and Waters

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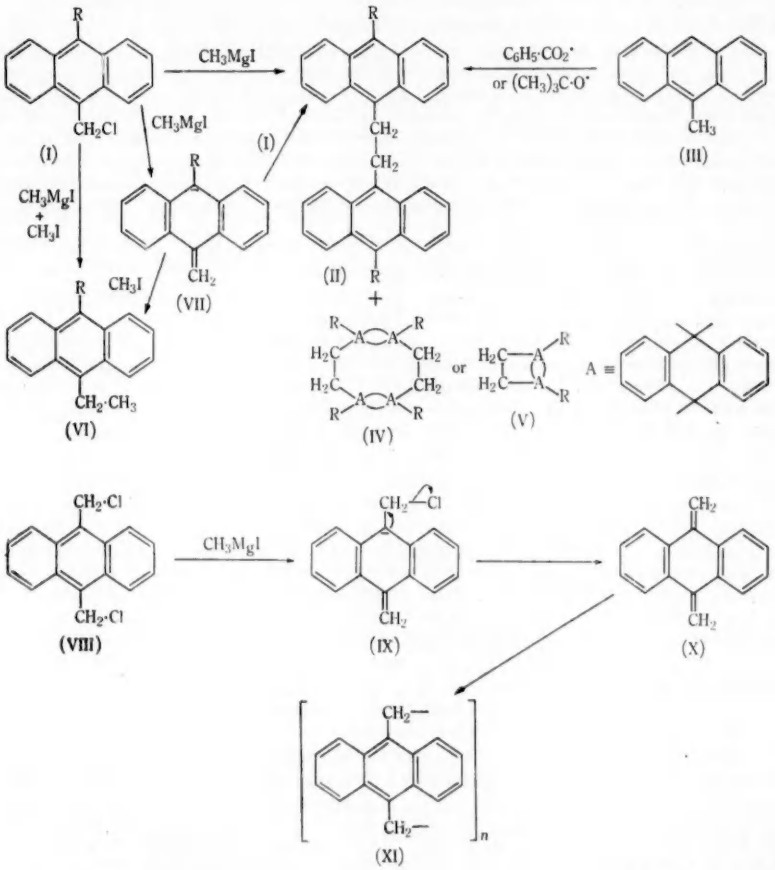
(1952)* and Beckwith and Waters (1956), who obtained both these compounds by the action of free radicals on 9-methylanthracene (III). No 9-ethylanthracene could be detected.



A detailed study of the action of methylmagnesium iodide on the benzyl halides has been carried out by Fuson (1926), who found that ~25% of ethylbenzene was produced along with ~66% of dibenzyl.

Similar behaviour towards the Grignard reagent was observed with 9-n-butyl-10-chloromethylanthracene (I; $R=C_4H_9$) and 9-dodecyl-10-chloromethylanthracene (I; $R=C_{12}H_{25}$). In both cases the corresponding 1,2-dianthryl-

* It is noteworthy that the evidence presented by Roitt and Waters does not completely exclude the monomeric cyclobutane structure (V; R=H) for this compound (cf. Greene, Misrock, and Wolfe 1955).

ethane (II; R=C_4H_9 or $C_{12}H_{25}$) was obtained as the major product, with a much smaller amount of accompanying "photodimer". Treatment of 9,10-dichloromethylanthracene (VIII) with the Grignard reagent produced a chlorine-free, amorphous yellow solid which, from its general properties, is probably a polymeric mixture represented by structure (XI).

The formation of 1,2-dianthrylethanes (II) in the presence of Grignard reagent suggests that a resonance-stabilized anion (VII) is very readily produced by metal-halogen exchange, and this then reacts with more of the chloromethyl derivative (I). The latter step will be catalysed by traces of iodine inevitably present under the reaction conditions used (Bavin 1960). Alternatively, a free radical coupling involving the stabilized radical corresponding to (VII) may operate (Waters 1948). The ionic mechanism is supported by the observation that slow extraction of 9-methyl-10-chloromethylanthracene (I; R=CH_3) into the refluxing Grignard reagent in the presence of excess methyl iodide gave 9-methyl-10-ethylanthracene (VI; R=CH_3) as the main product.

In the case of 9,10-dichloromethylanthracene (VIII) the situation is somewhat different since the initial anion (IX), corresponding to (IV), should undergo the electron shifts indicated with formation of the quinomethane intermediate (X). This reaction sequence, with ultimate formation of a polymeric material (as XI), corresponds exactly to the production of poly-*p*-xylylene by the action of metals on *p*-xylylene dihalides (Mann and Stewart 1954).

An interesting feature of the reaction of the chloromethyl compounds (I) with the Grignard reagent is the consistent formation of small amounts of the dimers as byproducts under essentially ionic reaction conditions. This dimerization of the dianthrylethanes (II) is probably promoted to some extent by the traces of iodine present.

Experimental

The microanalyses were carried out by the Australian Microanalytical Service, C.S.I.R.O. and University of Melbourne. All melting points are uncorrected.

The chloromethylanthracenes (I; R=H, C_4H_9, $C_{12}H_{25}$) have been described previously (Stewart 1960). 9,10-Dichloromethylanthracene was prepared by the method of Gudriniece and Vanags (1956), and 9-methyl-10-chloromethylanthracene according to Badger and Pearce (1950). In this last preparation a small quantity of *di-(9-methyl-10-anthryl)methane* was isolated as a sparingly soluble byproduct; yellow needles from toluene, m.p. 330–331 °C. For analysis it was recrystallized from toluene, and then from much benzene (Found: C, 93·7; H, 6·2%. Calc. for $C_{31}H_{24}$: C, 93·9; H, 6·1%).

Action of Methylmagnesium Iodide on meso*-Chloromethylanthracenes*

In each case the Grignard reagent (2–3 m-equiv.) was prepared in the normal way in ether from methyl iodide, magnesium turnings, and a small crystal of iodine. The chloromethyl compound was added at room temperature in benzene solution or as solid according to its solubility. In the latter case some benzene was added separately. The behaviour of each compound will be described individually.

(*a*) *9-Chloromethylanthracene* (*I*; *R*=*H*).—A heavy precipitate of 1,2-di-9-anthrylethane (II; R=H) separated a few seconds after the addition of a benzene solution of the chloro compound (0·5 g). The mixture was refluxed for an hour, decomposed with water, and dil. H_2SO_4 in the usual way, and filtered. The yield of crude product was 0·33 g (78%). It was recrystallized from benzene (sparingly soluble) and then from toluene when it formed faintly

yellow needles, m.p. 321–322·5 °C (decomp.), Roitt and Waters (1952) give m.p. 310–315 °C (decomp.), and Beckwith and Waters (1956) give m.p. 310 °C. The toluene mother liquor was kept at 0 °C for some time when a small quantity of the dimer described by Beckwith and Waters (1956) separated as colourless crystals having the same melting point as the parent compound.

Examination of the ethereal layer after filtration yielded some more of the compound (II ; R=H) and a trace of oily material.

(*b*) *9-n-Butyl-10-chloromethylanthracene* (*I* ; *R*=C_4H_9).—On addition of a benzene solution of the compound (0·5 g) a copious precipitate formed. The mixture was treated as described in (*a*) when 0·3 g(70%) of crude 1,2-*di*-(*9-n-butyl-10-anthryl*)*ethane* (*II* ; *R*=C_4H_9) was obtained. Recrystallization from light petroleum (b.p. 60–80 °C) gave light yellow needles, m.p. 202–203 °C. For analysis it was again recrystallized from light petroleum, m.p. 203–204 °C (Found : C, 92·3 ; H, 7·7%. Calc. for $C_{38}H_{38}$: C, 92·3 ; H, 7·7%).

A quantity (~10 mg) of the *dimer* was also produced, and was readily separated owing to its low solubility in light petroleum. It formed colourless needles from benzene–ethanol, m.p. 265·5–266 °C (decomp.) (Found : C, 92·1 ; H, 7·7%. Calc. for $C_{76}H_{76}$: C, 92·3 ; H, 7·7%). The same material was also obtained by exposing a dilute solution of the dianthrylethane (II ; R=C_4H_9) to sunlight until the fluorescence had disappeared. After evaporating to dryness, and washing the residue with methanol, the dimer was obtained, m.p. 265–266 °C (alone and mixed).

(*c*) *9-Dodecyl-10-chloromethylanthracene* (*I* ; *R*=$C_{12}H_{25}$).—As in (*a*) and (*b*) addition of the chloro compound (0·5 g) gave a crystalline precipitate and the development of blue fluorescence in the solution. Working up in the usual way gave 1,2-*di*-(*9-dodecyl-10-anthryl*)*ethane* (*II* ; *R*=$C_{12}H_{25}$) as yellow needles from light petroleum (b.p. 80–100 °C). The compound did not give a sharp melting point with meniscus, but shrank to a homogeneous mass at 135–137 °C. Chromatography on alumina gave a single fluorescent band (Found : C, 90·7 ; H, 9·8%. Calc. for $C_{54}H_{70}$: C, 90·2 ; H, 9·8%).

The corresponding colourless *dimer* (~26 mg) remained on the filter, and was recrystallized from benzene–ethanol, m.p. 201·5–202·5 °C (Found : C, 90·4 ; H, 9·8%. Calc. for $C_{108}H_{140}$: C, 90·2 ; H, 9·8%).

(*d*) *9,10-Dichloromethylanthracene* (*VIII*).—The sparingly soluble chloro compound (1·0 g) was refluxed with the Grignard solution (containing added benzene) for 3–4 hr, and the mixture decomposed in the usual way. A light yellow amorphous powder was obtained which was washed thoroughly with boiling ethanol. Yield 0·65 g (87% as polymer). This material was virtually insoluble in all the usual solvents (blue fluorescence was observed in the supernatant liquid in each case). It did not contain halogen, and left a slight ash on combustion. For analysis it was boiled with dil. HCl containing a little anionic detergent, and then washed with water and ethanol (Found : C, 88·8 ; H, 6·4 ; O, 2·3 ; ash, 1·0%. (Calc. for $(C_{16}H_{12})_n$: C, 94·1 ; H, 5·9%).

(*e*) *9-Methyl-10-chloromethylanthracene* (*I* ; *R*=CH_3).—The chloromethyl compound (1·2 g) was extracted into the refluxing Grignard solution containing an excess of methyl iodide. An almost clear solution was obtained, which, on working up, yielded 1·0 g (91%) of crude 9-methyl-10-ethylanthracene (VI ; R=CH_3). Recrystallization from ethanol, followed by chromatography on alumina and a final recrystallization gave the pure compound as light yellow needles, m.p. 143–144 °C (Found : C, 92·5 ; H, 7·3%. Calc. for $C_{17}H_{16}$: C, 92·7 ; H, 7·3%). Berliner (1944) gives m.p. 143–144 °C.

A small amount (50 mg) of unidentified yellow crystalline material remained on the filter, m.p. 306–307 °C (indefinite) (Found : C, 92·4 ; H, 6·1%). This material does not appear to be 1,2-di (9-methyl-10-anthryl)ethane (II ; R=CH_3) (see below).

A small-scale experiment with the chloro compound (I ; R=CH_3) in the presence of methylmagnesium iodide alone gave 1,2-di-(9-methyl-10-anthryl)ethane (II ; R=CH_3) as yellow needles from benzene–ethanol, m.p. 263–265 °C (decomp.) (Found : C, 93·3 ; H, 6·5%. Calc. for $C_{32}H_{26}$: C, 93·6 ; H, 6·4%). Beckwith and Waters (1956) give m.p. 272 °C. A trace of colourless crystalline material, presumably the corresponding dimer, was also formed.

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CORRIGENDUM

VOLUME 13, NUMBER 4

Page 459: In Table 1 *for* 3541 in column 2 opposite 1-Nitro-2-naphthylamine *read* 3514. The force constant k was calculated from this figure and no alteration is required. The authors are indebted to Professor A. N. Hambly for noting the error.

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